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Chronic Circadian Misalignment Leads to Reduced Longevity and Largescale Changes in Gene Expression in Drosophila Melanogaster

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CHRONIC CIRCADIAN MISALIGNMENT LEADS TO REDUCED LONGEVITY AND LARGESCALE CHANGES IN GENE EXPRESSION IN DROSOPHILA MELANOGASTER

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ABSTRACT

As a result of earth's orientation toward the sun producing day and night, organisms have evolved an endogenous circadian timing system that is responsible for the 24-hour oscillation of most physiological and behavioral processes. This timing system is constantly synchronized to the external environment to adapt to and anticipate changes in light, temperature, food, and mate availability. In modern society, social and work constraints cause people to live schedules that are out of sync with their internal circadian clocks, producing a chronic circadian misalignment (CCM). While epidemiological studies in humans point to potentially damaging metabolic and cognitive consequences of CCM, the full extent of these negative effects is unknown. Furthermore, very little is known about the molecular and cellular mechanisms that lead to the negative effects. Here, we model and investigate the consequences of CCM in the powerful model system of the fruit fly, *Drosophila melanogaster*, by exposing the flies to a 28-hour day comprised of 14-hours of light and 14-hours of dark (compared to control flies that are exposed to a standard 24-hour day). Consistent with previous results, we demonstrate that exposure of flies to the 28-hour schedule led to a 14.78% reduction in median lifespan in the females and a 14.72% reduction in males. Previously, it was unknown whether the reduced longevity that results from CCM is due to direct effects of circadian misalignment or whether it occurs secondary to changes in overall sleep or activity levels of misaligned flies. To differentiate between these possibilities, we used the Drosophila Activity Monitoring (DAM) system tocontinuously monitor fly locomotor activity and sleep while simultaneously conducting our

longevity analysis. This allowed us to assess the effect of long-term CCM on aging-associatedchanges in locomotor activity and sleep levels, and to correlate these measures with fly lifespan. While misaligned flies exhibited aberrant patterns of locomotor activity, evidenced by reduced rest:activity rhythm strength, overall sleep and activity levels were largely unchanged. Furthermore, the CCM-induced reduction in longevity persisted when we matched flies for sleep and activity levels, indicating that the reduction in lifespan was independent of these behaviors. To uncover potential molecular mechanisms of CCM-induced reduction in lifespan, we conducted whole body RNA-sequencing to assess differences in gene transcription between control and misaligned flies. Through this analysis, we identified several groups of genes that displayed altered expression under CCM conditions. These include upregulation of genes associated with cellular stress and downregulation of genes involved in the nervous system. This indicates that CCM induces endogenous stress in animals, potentially leading to reduced neuronal function.

INTRODUCTION

History and Early Work

The Earth's rotation and orientation toward the sun produce daily periods of light and dark which repeat every 24-hours. As a result, organisms have evolved endogenous timekeeping systems that enable them to anticipate such environmental changes instead of simply reacting to them. Because of this, most behavioral and physiological processes oscillate depending on the time of day. Jean Jacques d'Ortous de Mairan, a geophysicist and astronomer, was one of the first to study this phenomenon in a specific plant model, *mimosa pudica*. Mairan observed that plant leaves would raise and fall every day at specific times. While it was previously believed that this was simply the plant's reaction to sunlight, Mairan noticed these same daily rhythms occurred even in the absence of environmental cues (De Mairan, 1729). In the 1900s, chronobiology expanded and grew through work done by Jürgen Walther Ludwig Ashoff, a German physician, biologist, and behavioral physiologist. Ashoff's early work began through self-experimentation, in which he identified his own body's 24-hour rhythm in temperature (Daan and Gwinner, 1998). This work was continued by others including physiologist Nathaniel Kleitman. During his research, Kleitman subjected himself and another individual to Kentucky's Mammoth Cave, a location that was shielded from environmental lighting cues (Kleitman, 1963). Despite the use of lamps to self-impose a 28-hour lighting cycle, both Kleitman and the subject displayed normal rhythmic body temperatures that oscillated near a 24-hour fashion following one month's time in the cave. These studies contributed to early evidence uncovering

the innate endogenous circadian timing system which allows for the rhythmic, 24-hour oscillation of behavioral and physiological processes in nearly all organisms. These processes we now refer to as circadian rhythms (circa meaning "around", diem meaning "day").

Molecular Clock

Growing evidence of this endogenous circadian timing system and the identification of circadian rhythms lead to its investigation at the molecular level. Much of this work and current research today involves the model organism, *Drosophila melanogaster*. For over a century, *Drosophila*, or more commonly referred to as the "fruit fly" has proven to be one of the more useful model organisms to study behavior, physiology, and human diseases. This is due to several factors, including its short life cycle, ability to produce large quantities of offspring at a high rate, ease of maintenance, and its fully sequenced genome leading to simplicity in genetic manipulations (Hales et al, 2015). Through the years of circadian research, the fruit fly has been found to display robust rhythmic behaviors, including locomotor activity rhythms and eclosion rates (Tataroglu and Emery, 2014). Fly locomotor activity rhythms involve two peaks in activity during morning and evening hours, along with a siesta in the afternoon. In addition, flies begin to ramp-up their activity in anticipation to the lighting transitions. Similar patterns are seen in fly eclosion rhythms, in which a high rate of flies emerge from their pupae case during earlier hours, followed by a decrease in the afternoon and evening. These rhythms were also studied in constant darkness (DD) to allow for the behavior to free run in the absence of environmental influence. As both locomotor activity and eclosion maintained circadian rhythmicity, it became evident that an endogenous mechanism was present. Furthermore, these simple yet robust circadian rhythms became a useful tool in investigating the underlying molecular mechanisms dictating such behaviors.

In one of the earliest studies to investigate such molecular mechanisms, Konopka and Benzer conducted mutagenesis on flies and screened for eclosion rhythms outside the normal 24-hour period (Konopka and Benzer, 1971). They reasoned that characterizing mutants that expressed rhythms outside the 24-hour pattern would identify genes involved in the circadian mechanism. Through this screen, they identified three independent mutants with aberrant rhythms. Genetic mapping determined that these phenotypes were the result of different mutations of a single gene, which they named *period* (*per*). The first mutant expressed arrhythmic eclosion rhythms, (per^0); the second mutant expressed rhythmic behaviors, but with a 19-hour period (per^S); the third mutant also expressed rhythmic behaviors, but with a 28-hour period (per^L). It was also later determined that the protein it transcribes for, PERIOD (PER), undergoes robust circadian oscillation, suggesting its role in dictating behavioral rhythms (Zerr et al, 1990). This research was groundbreaking in that it identified and characterized the first clock gene and protein involved in the endogenous circadian timing system. Subsequent studies involved cloning of these clock genes, including *per* (Hall, 1995), now available and frequently used in chronobiogical research.

Over 20 years later, a breakthrough in circadian research was made by the identification of a second clock gene, *timeless* (*tim*) (Sehgal et al, 1994). Similar to previous work, a mutagenesis screen was performed to identify mutants with aberrant eclosion rhythms. Presence of arrhythmicity in eclosion rates and locomotor activity in constant darkness (DD) lead to the discovery of the *tim* gene. This gene not only showed a circadian rhythm in expression, but its translated protein was found to function in conjunction with PER (Vosshall et al, 1994).

Around the same time, Vitaterna et al. conducted a forward genetic screen in mice and identified the first mammalian clock gene, which they called mClock (mClk) (Vitaterna et al,

1994). The subsequent demonstration that mutations in the Drosophila homolog (*dClk*) produced a similar arrhythmic phenotype provided evidence that the circadian mechanism was well conserved. Importantly, *dClk* was shown to regulate *per* and *tim* levels in flies (Allada et al, 1998). This influence was explained through the discovery of a fourth clock protein, CYCLE (CYC), which together with CLOCK (CLK) forms a heterodimer that binds to *per* and *tim* promoter E-box (Rutila et al, 1998) As *per* and *tim* proteins were later confirmed to function as heterodimers to inhibit transcription of CLK:CYC (Darlington et al, 1998), the formation of the negative feedback loop model explaining the interaction of these four clock proteins came to fruition.

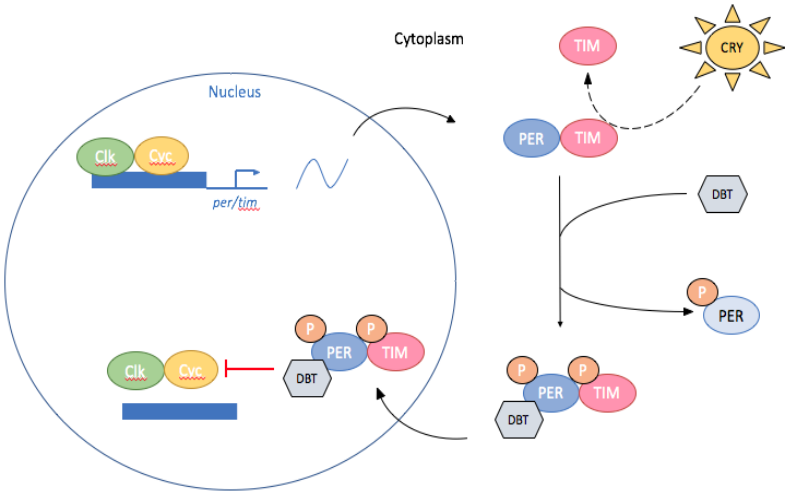
In summary, this mechanism begins by CLK/CYC heterodimer binding to a E-box promoter region, driving transcription of *per* and *tim* in the nucleus during morning hours. These genes are then translated in the cytoplasm, allowing PER and TIM proteins to accumulate and dimerize during evening hours. PER/TIM are then phosphorylated by a number of different kinases, including DOUBLETIME (DBT) (Price et al, 1998), which regulate their degradation and nuclear entry. This kinase regulation is absolutely essential to ensure that the molecular clock cycles with a ~24-hr period. Accumulated PER/TIM containing bound DBT then re-enters the nucleus and binds to CLK/CYC, inhibiting its function at around midnight. This inhibition leads to the reduction and degradation of PER and TIM levels, closing the negative feedback loop and resetting the mechanism. (Allada and Chung, 2010). In addition to regulating *per* and *tim* transcription, the CLOCK/CYCLE complex also regulates the expression of thousands of other gene targets, thus establishing rhythmic expression of many genes involved in various functions in the cell. These targets are especially involved in pathways leading to locomotor activity rhythms and eclosion rates.

Entrainment and Input Pathways

While the development of the negative feedback loop mechanism began to explain the oscillation and rhythmicity of certain behaviors during specific times of day, the understanding of how circadian timing systems synchronizes to the external environmental cues was lacking. This gap in our knowledge was filled through the identification of Cryptochrome (CRY). Early work found evidence to suggests its role as photoreceptor in specific tissues of the brain that is necessary for the entrainment and maintenance of the circadian rhythms (Emery et al, 1998; Emery et al, 2000). Today, we know that CRY functions as a photoreceptor that binds and degrades TIM when activated, resetting and synchronizing the phase of the negative feedback loop mechanism within specific neurons of the fly brain (Allada and Chung, 2010). Furthermore, we know that CRY, as well as the other molecular clock components, are also expressed in peripheral tissues, explaining the ability of many of these peripheral tissues to entrain to environmental cues independent of the brain. These mechanisms compose the flies input pathways, in which their endogenous clock can use external information to entrain their endogenous circadian timing system.

Taken together, we see the mechanisms dictating the molecular clock is the product of 6 core proteins: PER, TIM, CLK, CYC, DBT, and CRY. Through their interaction and coordination, these proteins produce the oscillatory mechanism that dictates circadian rhythms seen in biochemical, physiological, and behavioral pathways (**figure 1**).

Figure 1. The molecular negative feedback loop mechanism



CLK and CYC form a heterodimer and bind to a specific E-box that drives transcription of *per* and *tim*. Translated proteins PER and TIM are then phosphorylated by kinase phosphorylation (e.g. DBT), which regulates protein levels and nuclear localization. PER and TIM accumulation causes their binding and entrance into the nucleus, further inhibiting the function of CLK/CYC. Photoreceptor CRY is activated by light and causes degradation of TIM, resetting and entraining this mechanism to the environmental cues.

Core Clock Neurons

As the details of the molecular clock mechanism were uncovered, researchers became interested in its location and coordination within the *Drosophila* CNS. This research began in a study that transplanted *Drosophila* brains of short-period (per^S) mutants to arrhythmic (per^o) mutant hosts to determine if a phenotypic rescue occurred (Handler and Konopka, 1979). Following this procedure, they found that per^o flies expressed the phase in activity rhythms of the donor, in this case per^S flies. This, combined with similar results found in later mammalian studies (Ralph et al, 1990), provided early evidence that the brain acts as the central pacemaker controlling the circadian timing system. An important turning point in this work occurred when researchers chose to use immunohistochemistry and *in situ* hybridization to identify cells in the brain that express the clock genes. Today, it is understood that the fly brain contains ~150

neurons that are responsible for controlling circadian rhythmicity. These clock cells are categorized into three subcategories, which include small and large ventral lateral neurons (sLN_v and lLN_v), the dorsal lateral neurons (LN_d), and the three types of dorsal neurons (DN1, DN2, and DN3) (Nitabach and Taghert, 2008). These neurons, containing the molecular mechanism which maintain circadian rhythmicity, make up the core clock of the circadian timing system located throughout the fly brain. In mammals, a homologous set of core clock neurons are located in the suprachiasmatic nucleus (SCN) (Landgraf et al, 2014).

Once the different groups of clock cells were identified, researchers began to investigate whether different subsets of clock neurons played unique roles in establishing behavioral rhythms. This began in work that found *per* expression in certain locations of the brain to be more important for driving circadian rhythmicity compared to others, specifically those containing LNs (Ewer et al, 1992). Through the use of a *disco* mutant, which lack LNs, others confirmed the presence of just one ventral lateral neuron (LN_v) to be enough to maintain normal activity rhythms, identifying a specific group of neurons involved in maintaining circadian rhythmicity (Helfrich-Förster, 1998). Shortly following, a second subset of cells were identified that appeared to share this role in the circadian timing system, being the dorsal lateral neurons (LN_ds) (Stoleru et al, 2004). Here, researchers found that while the LN_vs were required for morning anticipation in activity, LN_ds were needed for evening anticipation. Furthermore, subsequent research indicating clock restoration in LN_ds associated with the rescue of evening anticipation confirmed these results (Grima et al, 2004). Other work has also pointed to an important contribution of the dorsal neurons (DN1s) that appeared to function in maintaining behavioral rhythms at specific times of day (Murad et al, 2007). These studies, among many others, have contributed to our current, more in-depth understanding of the different roles of each

clock group within the *Drosophila* brain (Dubowy et al, 2017).

Output pathways

Though the clock neurons are able to keep time independently, in order to produce rhythmic behavioral and physiological processes they must be connected to downstream brain regions, referred to as output pathways. These neuronal networks are subject to several ongoing studies that look to map the specific constituents involved in eliciting circadian behaviors signaled by the core clock. Important advancements in this field involve the work done by Cavanaugh et al, in which GFP reconstitution across synaptic partners (GRASP) identified a functional connection between core clock neurons and cells of the pars intercerebralis (PI) (Cavanaugh et al, 2014). Continuing to map this pathway, King et al later determined that these PI cells connect to hugin+ SEZ neurons, which then extend to the ventral nerve cord to control locomotor activity rhythms (King et al, 2017). Another example of this involves work by Cavey et al, in which they identified the connection between core clock neurons and a Leucokinin neuropeptide circuit, as well as DH44-expressing neurons (Cavey et al, 2016). Together, these studies represent recent hallmark findings in the pursuit of mapping the output pathways linked to the core clock.

In summary, the fruit fly contains an endogenous circadian timing system composed of input pathways, a core clock, and output pathways which function in harmony with one another to produce behavioral and physiological rhythms. It is through the ability to synchronize to the environment where we are able to see this system functioning correspondently with the natural world.

Chronic Circadian Misalignment (Humans)

While the circadian timing system synchronizes to cycles of light and dark, this process is not immediate. If placed in altered lighting conditions, the timing system must re-synchronize to the new schedule, producing a period of misalignment (or desynchronization). Due to social and work constraints, humans subject themselves to environmental cues (typically lighting) that are out of synchrony with their endogenous clock. This creates misalignment between the endogenous rhythms of core clock neurons in the SCN and the external environmental cues, as well as the discoordination of SCN rhythms and peripheral tissue rhythms. If this misalignment is maintained and repeated over a prolonged period, the condition is referred to as chronic circadian misalignment (CCM).

CCM has become common in modern society and is associated with negative health effects. A growing number of epidemiological studies have shown that people experiencing CCM are prone to developing different diseases, disorders, and physiological and behavioral aberrations. CCM is especially prevalent in careers involving frequent transmeridian travel (such as pilots and flight attendants). Flight attendants and pilots must travel through different time zones on a weekly basis, requiring exposure to varying lighting schedules. In doing so, these people are experiencing a form of CCM commonly known as chronic jetlag. As a result, researchers have found these people to be at a higher risk of developing malignant melanoma, breast cancer, spontaneous abortions, and cognitive deficits (Tokumaru et al, 2006; Stevens, 2009; Aspholm et al, 1999; Cho et al, 2000). Chronic circadian misalignment is also very common in those practicing shift work, which involves work outside the typical 9:00am-5:00pm workday. This includes those working night shifts or work schedules that change throughout the week. Combined with social obligations, these workers don’t allow the body to properly align to

the different lighting schedules. Similar to flight attendants and pilots, shift work is associated with a number of different physiological changes and pathological disorders. A study that exemplifies this identified changes in melatonin levels and elevated sleep disruptions in shift workers (Bursch et al, 2005). Others have also identified an association between night shift workers and the incidence of breast cancer (Schernhammer et al 2006; Stevens, 2009). Furthermore, an epidemiological review of this work indicates reoccurring themes of gastrointestinal and cardiovascular disorders following exposure to shift work (Costa, 1996). Taken together, these studies suggest that CCM increases the risk of negative health effects in humans.

CCM is not restricted to these select occupations. In fact, many individuals follow irregular schedules and thus expose themselves to CCM. This results in what has been termed social jetlag, which is a form of circadian misalignment that is brought upon when individuals sleep and wake during times that are not in sync with their circadian timing system. An example of this includes students who have a specific sleep-wake schedule during the weekday, but then stay out and sleep in late during the weekend. Like chronic jetlag, social jetlag has also been found to produce CCM, resulting in negative health effects such as cognitive deficits and memory loss in students and other individuals (Wittmann et al, 2009; Lau et al, 2013).

While these epidemiological studies indicated a potential connection between CCM and negative health effects, an assessment of cause and effect was lacking. This lead researchers to begin conducting controlled lab studies in human subjects to uncover the underlying mechanisms behind CCM and its associated effects. One study that did so subjected 10 individuals to either a control 24-hour day (12 hours light:12 hours dark) or a 28-hour day (14 hours light: 14-hour dark) (Scheer et al, 2008). During and after the 10-day experiment, subjects exposed to the 28-

hour day experienced several metabolic and cardiovascular changes, all of which known to be precursors to more harmful disorders. These include changes is metabolic and stress hormones, increased mean arterial pressure, reduced sleep efficiency, and prediabetic symptoms in 3 of the 10 individuals. These effects, combined with the aforementioned epidemiological studies, indicate the consequences and relevancy of CCM.

Chronic Circadian Misalignment (Mammalian Models)

In an attempt to further assess the effects and underlying mechanisms behind CCM, researchers have developed animal models that have allowed for a more in depth analysis (Golombek et al, 2013). In one of the earlier studies, researchers exposed mice to three different lighting schedules. This included a control 12:12 light-dark schedule and either a 6-hour phase advance or phase delay every seven days. Under such conditions, aged mice expressed a profound decrease in lifespan under phase advancing conditions compared to control conditions. To further test this effect, they increased shift frequency to every four days instead of seven, which lead to an even greater reduction in longevity (Davidson et al, 2006). Due to the large amount of epidemiological studies indicating an association between cancer incidence in humans and CCM, studies also began assessing tumorigenesis in mammalian species under similar conditions. In one set of experiments, researchers subjected mice to either a control 12:12 light-dark schedule or an 8-hour phase advance every 2 days following inoculation of Glasgow osteosarcoma, and found that tumors grew faster in the phase advancing mice compared to the controls (Filipski et al, 2004). This effect they attributed to disrupted clock gene expression, such as mPer2, which displays anti-tumor growth properties (Fu et al, 2002). These studies, and many others, indicate that model organisms produce consistent adverse effects when experiencing CCM. Additional examples of these include accelerated aging, increased weight gain, and

cognitive deficits (Vinogradova et al, 2010; Fonken et al, 2010; Gibson et al, 2010). While these studies have enlightened scientists and the public of these negative consequences, researchers continue to push this work forward in invertebrate models.

Chronic Circadian Misalignment (*Drosophila melanogaster*)

In addition to these mammalian studies, researchers have also modeled CCM in fruit flies to utilize the many benefits and advantages mentioned previously. In early work, predating most mammalian studies, researchers exposed flies to 4 different environmental conditions: a control 24-hour day (12 hours light:12 hours dark), a 21-hour day (10.5 hours light:10.5 hours dark), a 27-hour day (13.5 hours light:13.5 hours dark), or constant light, and found that flies exposed to altered environmental conditions expressed a reduction in lifespan compared to the control 24-hour day (Pittendrigh and Minis, 1972). Others conducted a similar study in which they identified specific fly lines that experienced a reduction in longevity when exposed to a random light-dark regime (RLD) (Ringo et al, 1986). Both studies attributed the reduced lifespans to a lack of resonance between the endogenous period of the animal and the environmental cycle. More recently, researchers conducted a study to further characterize the effects of CCM. Here, they assessed locomotor aging and longevity in flies containing genetically or environmentally disrupted circadian timing systems (Vaccaro et al, 2016). Two *period* gene mutant animals were compared: per^{01} which eliminates circadian rhythms, and per^{T} which expressed 16-hour endogenous rhythms. When these flies were exposed to a 24-hour light:dark schedule, the per^{T} mutants had reduced longevity and decreased startle-induced locomotion (accelerated locomotor aging) when compared to wild-type flies. The change in startle induced locomotion was identified using the SING assay, in which the amount of time it took the flies to climb up a vial after being startled was impaired. When these flies were then placed in a 16-hour light:dark

schedule, wild-type flies now had a decrease in startle-induced locomotion, while they saw a rescue of this in the *per*^{T} mutants. This indicated that the reduction in health span of the fruit fly was the result of the misalignment of the endogenous circadian timing system to environmental cues, not a lack of overall health in mutant flies. Furthermore, this study paved the way for further assessment of the overall consequences of CCM. Due to the large number of behavioral and physiological processes in the fruit fly that are regulated by the circadian timing system, studying the effect of CCM on these various processes provides an opportunity to learn the extent of the harmful effects of CCM.

Research Aims

Prior to experimentation, we began this study by first creating a model for assessing CCM using *Drosophila melanogaster*. This was achieved by exposing the flies to a 28-hour light:dark "chronic jetlag" schedule (14-hours light; 14-hours dark), which does not allow for proper alignment of the flies' internal clock with the environmental cues. This brings us to the first goal of our study, which was to conduct a more in-depth analysis of the behavioral and physiological consequences of chronic jetlag. This involved observing locomotor activity and rhythmicity, sleep duration, and longevity simultaneously. The second goal of this study was to investigate the molecular changes brought about by CCM that could lead to negative health consequences such as reduced longevity. This was achieved through RNA sequencing and stress reporter lines, which indicates changes in gene expression associated with the effects of CCM.

CHAPTER ONE

ASSESSING THE CONSEQUENCES OF CHRONIC CIRCADIAN MISALIGNMENT

Central Hypothesis: Chronic circadian misalignment leads to an overall reduction in health and well-being in *Drosophila melanogaster*.

Specific Aim 1: Assess locomotor activity, sleep, and longevity in *Drosophila melanogaster* exposed to a jetlag schedule to determine if chronic circadian misalignment leads to changes in behavior and physiological health.

- Hypothesis: Exposure of flies to a 28-hour chronic jetlag schedule consisting of daily 4-hr phase delays will reduce locomotor activity rhythm strength and longevity compared to flies exposed to a normal 24-hour schedule.
- Approach: Use the DAM monitoring system to continuously measure locomotor activity while simultaneously assessing lifespan during exposure of flies to either a 28-hour chronic jetlag schedule or a 24-hour control schedule. This constant monitoring of fly locomotor activity will allow us to determine effects of chronic circadian misalignment on longevity, locomotor activity and sleep and to correlate changes in longevity with overall sleep and activity levels.

Background

Several studies have assessed the effects of CCM, produced through exposure to aberrant lighting schedules, by measuring changes in longevity. This involved work done by Pittendrigh

and Minis, who observed decreased longevity following chronic exposure to either a short, 21-hour day or a long, 27-hour day (Pittendrigh and Minis, 1972). Similarly, Ringo et al. exposed flies to a random light-dark regime (RLD) and found a subsequent reduction in longevity (Ringo et al, 1986). Building upon this, researchers have also investigated CCM through the use of clock gene mutations that either changed the endogenous period to reduce resonance with normal 24-hr lighting cues, or left flies completely arrhythmic. For example, under normal 24-hour conditions, both per^{T} mutants, which have extremely short endogenous periods, and per^{L} flies, which have long endogenous periods, have reduced longevities compared to the wild type (Klarsfeld and Rouyer, 1998). Subsequent work confirmed that per mutations shortened longevity, and further demonstrated that the negative consequences of short period mutations could be mitigated by raising flies under short period lighting regimes (Vaccaro et al, 2016). This, along with other research indicating reductions in longevities among other clock mutants (cyc^{0} and tim^{0}), has suggested the importance of alignment between clock and environmental cues in maintaining physiological health (Vaccaro et al, 2017).

Despite the effective use of longevity as a measure of overall health, it remains unclear as to why CCM is associated with reduced longevity and whether or not it is secondary to other behavioral changes such as sleep or activity levels. This uncertainty demands a more accurate assessment of such behaviors to fully characterize the effects of CCM on health. Here, we chose to simultaneously monitor fly locomotor rhythmicity, locomotor activity and sleep amount, and longevity while exposing flies to CCM.

To achieve a CCM schedule, we exposed flies to a 28-hour (14-hours light; 14-hours dark), chronic jetlag schedule. This was compared to a 24-hour (12-hour light; 12-hour dark), control schedule. We used the DAM monitoring system to quantify differences in locomotor

rhythmicity, locomotor activity, sleep amount, and longevity (further explained in methods section). In doing so, we demonstrate reduced median lifespan in both male and female flies exposed to our chronic jetlag schedule, consistent with previous results. We find that this occurs in the absence of obvious decrement in function of the core molecular clock. Finally, we demonstrate that despite the fact that CCM slightly reduces total sleep duration and increases activity levels (specifically in males), reduced longevity was independent of these behavioral changes.

Methods

Longevity Analysis

Male and female iso31 flies were collected within 2 days of eclosion. Individual flies were loaded into glass tubes containing a 5% sucrose/2% agar food source and placed in Drosophila Activity Monitors *(*DAMs). Humidity and temperature-controlled incubators were used to expose flies to either a 24-hour schedule (12-hours light, 12-hours dark; control group), or a 28-hour experimental schedule (14-hours light, 14-hours dark; chronic jetlag group). Incubator temperature was held constant at 25°C and humidity levels were kept between 70% and 80%. Flies were transferred to new tubes each week to supply fresh food. Locomotor activity of male and female flies was monitored using the Drosophila Activity Monitoring System (Trikenetics). DAMs contain an infrared beam shot directly through the center of each tube. Activity was recorded when the fly crossed the tube's midpoint and interrupted the beam. Longevity was determined by identifying the fly's last activity time in DAM data. Occasionally we observed "ghost" readings, where single beam breaks were detected even after flies had died. Thus, we removed an activity bin that was identified ≥ 12-hours after previous activity. Data were collected until all flies in the experiment were dead.

Locomotor Activity and Sleep Analysis

Analysis of locomotor activity was done with ClockLab software (Actimetrics). Rhythmicity of activity was determined by using X^2 periodogram analysis, which was done in 7 day bits to assess weekly locomotor rhythmicity. Sleep was identified and counted if 5 consecutive bins of inactivity occurred (Ho and Sehgal, 2005) as determined by a custom-developed Excel formula. For full life sleep, we removed the last three days from analysis because flies reduce activity during this time, making it difficult to separate sleep from an age-induced decrease in locomotor activity.

Results

CCM Reduces Lifespan

Fly longevity was initially assessed to determine large-scale consequences of CCM. **Figure 2** demonstrates that flies exposed to the 28-hour (jetlag) schedule experienced a reduction in longevity compared to those exposed to a 24-hour (control) schedule. Male jetlag flies had a 14.72% reduction in median longevity compared to male control flies (median longevity for jetlag males was 19.6 days compared to 23.0 days for controls; p=2.65e-07, LogRank Test). We observed similar results in females, in which jetlag flies had a 14.78% lifespan reduction compared to controls (20.2 days compared to 23.7 days; p=1.56e-04, LogRank Test). These results are consistent with those found in previous work (Pittendrigh and Minis, 1972; Vaccaro et al, 2016), confirming the consequential impact of CCM on physiological health.

CCM Causes Aberrant Locomotor Activity Patterns

We chose to house flies in DAMs for the duration of the experiment so that we could simultaneously assess locomotor activity. Male and female flies exposed to jetlag conditions

expressed aberrant locomotor activity patterns during each week of the experiment. More specifically, jetlag flies displayed early anticipation to lighting transitions during early weeks, and also seemed to lose their characteristic activity bout in transition to lights-off during later weeks, especially males (**fig. 3**).

Perhaps due to mistimed morning and evening anticipation, jetlagged flies also displayed reduced locomotor activity rhythm strength throughout the duration of the experiments, as seen in **figure 4**. While both experimental and control female flies showed natural reductions in locomotor rhythmicity as they aged ($F_{(3,652)} = 66.83$, p=0.000; 2-way ANOVA; main effect of time), jetlag flies overall expressed significantly reduced locomotor activity rhythm strength for the duration of the experiment compared to controls ($F_{(1,652)} = 28.93$, p=0.000; 2-way ANOVA; main effect of treatment). Similarly, jetlag male flies also exhibited reduced locomotor activity rhythm strength compared to controls ($F_{(1, 611)} = 25.64$, p=0.000; 2-way ANOVA; main effect of treatment).

This reduced rhythmicity could be due either to the misalignment between internal and external rhythms or due to CCM-induced damage to core clock neurons or molecular cycling. To test for the latter, we assessed locomotor rhythmicity of flies in DD following exposure to varying amounts of time in either jetlag or control conditions (**fig. 4C-D**). Our data suggest the central clock and associated output pathways maintain proper functionality following exposure to chronic jetlag. In male flies, no differences in rhythmicity were identified between control and jetlag groups ($F_{(1, 60)} =0.25$, p=0.616; 2-way ANOVA; main effect of treatment). Despite female jetlag flies appearing to display increased rhythmicity in DD compared to the controls ($F_{(1, 66)} = 5.48$, p=0.022; 2-way ANOVA; main effect of treatment), post hoc analysis found no statistical difference in rhythmicity between jetlag and control flies for any given week (Tukey's HSP

p>0.05; **fig. 4D**). The fact that jetlagged flies have normal rhythm strength in DD demonstrates a functional central clock and further suggests that the reduced rhythm strength observed under LD conditions is a result of the difference in the endogenous period of the fly and the environmental conditions.

CCM Decreases Longevity Independent of Changes in Locomotor Activity or Sleep Duration

While our results indicating a reduction in longevity among flies exposed to an aberrant lighting schedule are consistent with previous work (Pittendrigh and Minis, 1972; Ringo et al, 1986), how misalignment is affecting physiological health remains unknown. While we ruled out damage to molecular cycling and the core clock, one remaining possibility is that the CCM-inducing schedule elicit altered locomotor behaviors. In doing so, this could shift the metabolic output through elevated activity and reduced sleep, which have been found to result in reduced longevity in previous work (Bushey et al, 2010). To investigate whether changes in sleep or activity are causing the reduced lifespan, we began by initially determining whether sleep amount was correlated with longevity in our experiments. Interestingly, we found that sleep amount in male flies was positively correlated with longevity. For the control males, correlations were seen between lifespan and sleep in first week of life (p=0.000, rho=0.454; Spearman rank test), as well as between lifespan and total lifetime sleep (p=0.002, rho=0.269; Spearman rank test) (**fig. 5F, B**). This was also true in the jetlag males, in which the first week of life (p=0.000, rho=0.419; Spearman rank test) and total lifetime sleep (p=0.002, rho=0.275; Spearman rank test) displayed positive correlations with longevity (**fig. 5H, D**). In contrast, we found no correlative relationships between sleep and longevity in the female flies (**figure 5A, C, E, G**).

Due to this identified relationship between sleep and longevity in the males, we next sought to determine whether jetlagged flies had reduced sleep amount, which could potentially explain their early death. Interestingly, we found significant reductions in jetlag males during week 1 (mean of 44.72 ± 0.5 min sleep/hour compared to 47.71 ± 0.3 for controls), week 2 (mean of 39.17 ± 0.6 min sleep/hour compared to 44.11± 0.4 for controls), and the full lifetime (mean of 41.93 ± 0.5 min sleep/hour compared to 45.04 ± 0.3 for controls) (**fig. 6A-C**). The jetlag females only displayed reduced sleep amount during week 1 (**fig. 6B**). Due to sleep amount being a relative inverse of locomotion, we found similar differences between groups regarding locomotor activity amounts as well, in that jetlagged flies had increased activity (**fig. 6D-F**). Despite these differences, it is important to note that jetlag flies still obtain a substantial amount of daily sleep, and that the magnitude of reduction was less than 5 min. sleep/hour.

Because the correlation results indicated no relationship between sleep and longevity in female flies, we can conclude that the reduction in longevity seen in jetlag flies was not the result of the reduced sleep identified in week 1. However, male flies did show a correlation between sleep and longevity. Furthermore, jetlag flies displayed reduced sleep during whole life and week 1, indicating a potential factor effecting their reduced longevity. To address this, we compared the median longevities of flies matched for total sleep amount by pairing each control fly with a matching jetlag fly that exhibited an average sleep amount within 1 min. sleep/hour of the control fly. This enabled us to compare longevities between control and jetlag flies that had no statistical difference in sleep (p-value >0.05, 2-tailed t-test; fig. 7E, F). We determined that both male and female jetlag flies maintained their reduction in longevity compared to the controls even when sleep amounts were normalized (p-value <0.05, LogRank Test), although the magnitude of reduction in longevity was partially reduced in males (**fig. 7B, D**). This

demonstrated that the reduced longevity resulting from CCM is independent of the minor changes in activity and sleep that are associated with the 28-hour (chronic jetlag) schedule.

Discussion and Conclusions

Simply by exposing flies to a 28-hour (chronic jetlag) schedule, we see both males and females experience reduced longevities. Firstly, this phenotype confirms data from previous work, in which flies exposed to aberrant lighting schedules also display reductions in longevity (Pittendrigh and Minis, 1972; Ringo et al, 1986). Second, these results are in line those obtained from mammalian and human studies, in which different forms of CCM have been extensively shown to negatively affect health (Evans and Davidson, 2013), thus providing evidence for a conserved function of the circadian timing system across species.

While previous work has shown reductions to longevity in CCM-inducing environments, it was unknown whether this was a direct result of misalignment, or secondary to behavioral changes (such as altered locomotor activity and sleep behaviors) that are produced from such schedules. While some have assessed the effects of CCM on the climbing ability of flies (Vacarro et al, 2016), these experiments were conducted following exposure to CCM, not during. Thus, we chose to use the DAM monitoring system to, for the first time, simultaneously monitor locomotor activity and sleep behaviors of flies experiencing CCM.

Through these studies, we report several important findings. First, we found that flies exposed to chronic jetlag exhibited aberrant locomotor activity indicative of the need to continually phase shift their circadian clocks in order to remain synchronized to the 28-hour day. This was evidenced by the fact that jetlag flies expressed early anticipation to the lighting transitions, which is likely a result of the 2-hour delay to each lighting transition within the 28-hour schedule. However, flies displayed partial entrainment to the 28-hour schedule, seen

through the absence of a free running locomotor activity response. This suggests that the flies were most likely experiencing daily disruption and adjustment of their molecular clock as a result of the lack of resonance between endogenous and environmental rhythms. During later life flies also showed reduced activity bouts at lighting transitions, particularly in the males. Because flies have an innate reaction to lighting transitions, its absence in jetlag flies is indicative of reduced physiological health compared to controls.

Due to altered locomotor activity behaviors, we found that CCM reduced locomotor rhythm strength in LD conditions in both male and female jetlag flies compared to controls. When these flies were then placed in DD, no changes in rhythmicity were seen between control and jetlag groups when comparing each week. This determined that the endogenous clock of flies exposed to chronic jetlag was still functioning properly, and that direct damage to core clock neurons or molecular cycling had not occurred. This also suggested that the reduction in longevity was more likely the result of the misalignment between the internal clock and the external environment.

Second, we discovered a positive correlation between sleep duration and longevity in both control and jetlag males, while no relationship was identified in the females. These results suggest a higher level of importance of sleep in male flies compared to females, specifically during early life. While other studies have also identified an effect of sleep changes on longevity (Bushey et al, 2010), none have identified or addressed the differences between males and females. Subsequent work focusing on these differences may help explain the effect of gender on sleep disorders in humans (Mallampalli et al, 2014).

Third, we demonstrated that chronic jetlag using a daily phase-delay paradigm causes reduced sleep, specifically in the males. Sleep aberrations in the jetlag flies are likely due to early

and sustained anticipatory increases in activity prior to lights on and off. Furthermore, jetlag flies have 2 peaks in activity during evening hours. The first occurs as flies anticipate lights-off near ZT12, and the second by the startle response induced by the lights turning off 2 hours later, potentially producing some level of sleep disruption (**fig. 3C and D**). Because sleep was calculated using the activity data, we identified similar results when quantifying locomotor activity amounts (**fig. 6**).

Finally, we indicate that the reduced longevity that results from CCM occurs independent of changes in sleep or activity among both sexes. Our sleep matching analysis accounted for this, in which we showed that chronic jetlag flies experiencing the same amount of sleep as controls maintained a reduction in longevity. Furthermore, we determined that these flies selected also had no differences in activity. However, we did find that the lifespan reduction was slightly decreased in the males after accounting for changes in sleep amount. This may suggest the behavioral changes may be an additional consequence of CCM in the males, but not the determining factor producing reduced longevity. Nevertheless, this data indicates that CCM, and not minor changes in sleep and activity, produced the reduction in longevity. The mechanism behind this phenotype was investigated in our second aim, which is discussed in chapter 2.

Overall, our results are consistent with both mammalian and human epidemiological studies (Golombek et al, 2013; Costa, 1996). The reduction in longevity independent of changes in sleep or activity have large implications to human behavior, in that those experiencing chronic jetlag may not be alleviating themselves from the negative consequences by simply increasing sleep amount. For those frequently experiencing CCM (through social and occupational obligations), this stresses the importance to seek light therapeutics to achieve resonance of endogenous and external clocks.

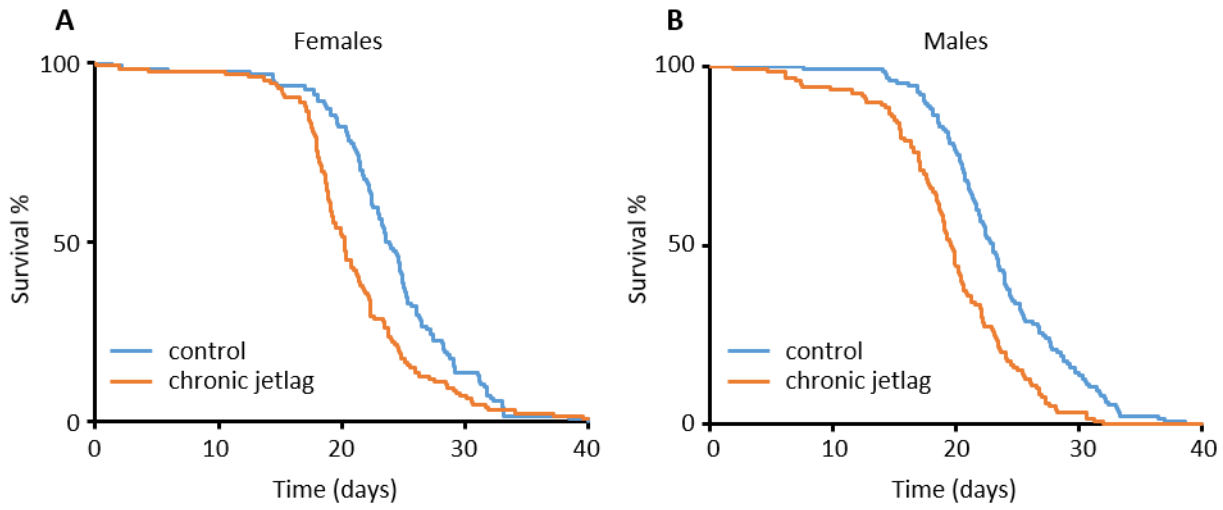
Future Directions

In future experiments, we will conduct additional behavioral assays to fully characterize the consequences of CCM. The first involves an assessment of fly cognition and memory. The circadian timing system is known to govern the neural circuits involved in learning and memory (Smarr et al, 2015), providing an opportunity to determine another physiological process effected by CCM. In previous epidemiological studies, CCM has been associated with cognitive disorders and memory deficits (Cho et al, 2000). While similar results have been indicated in mammalian studies (Loh et al, 2010), little to no work has been done involving the fruit fly. We will use olfactory conditioning paradigms (Malik and Hodge, 2014) to determine if flies exposed to chronic jetlag experience subsequent changes in learning and memory.

We are also very interested in determining the consequences of social jetlag, which occurs when individuals follow irregular sleep/wake cycles, resulting in negative health effects (Wittmann et al, 2009; Lau et al, 2013). We will study the effects of social jetlag in fruit flies by exposing them to a 9am to 9pm LD schedule during the week (Monday through Thursday), followed by a 1am to 1pm LD schedule during the weekend (Friday through Sunday). In doing so, we can determine if this form of CCM produces similar changes in health span of the fruit fly. Furthermore, we can conduct memory assays under social jetlag conditions as well.

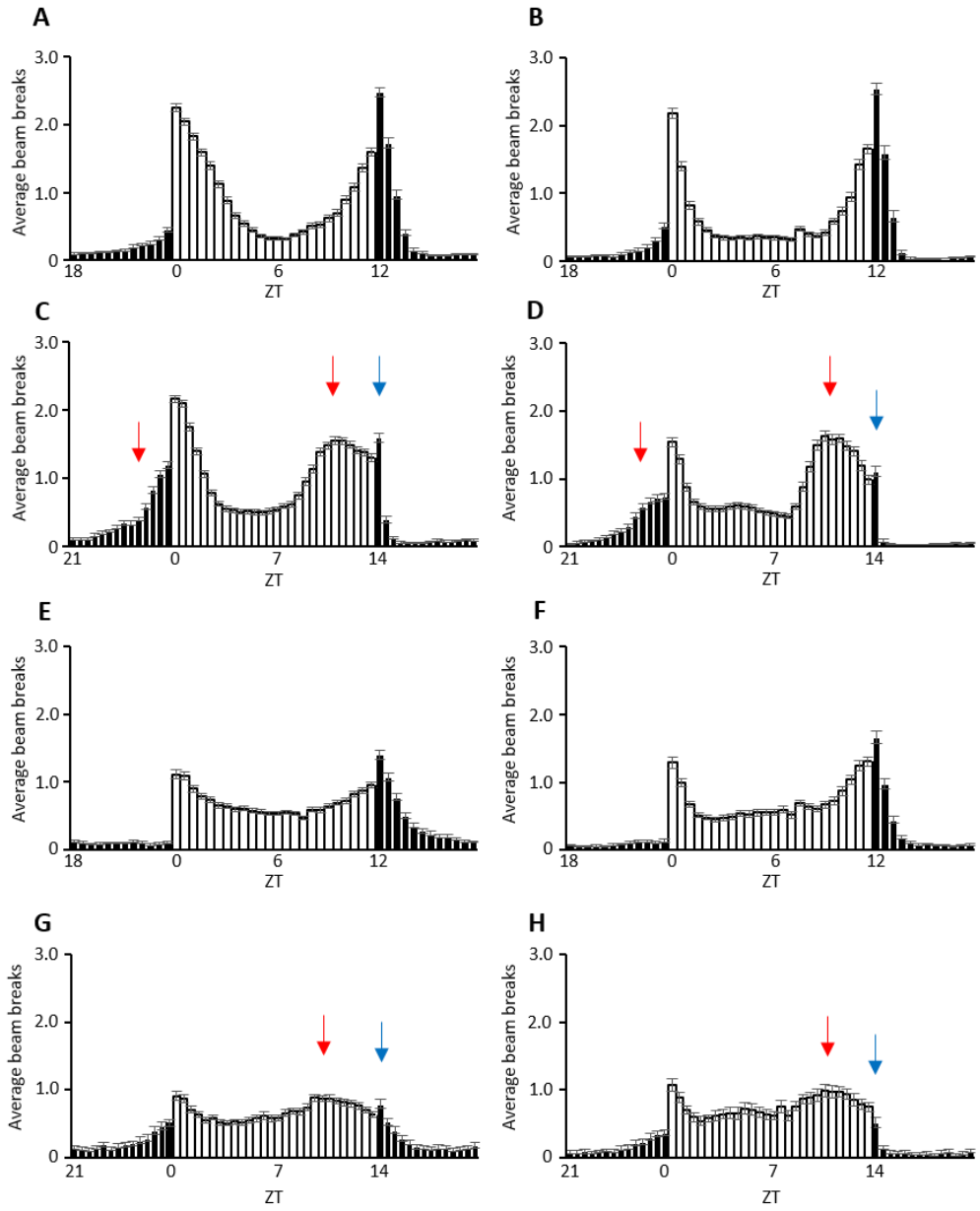
Figures

Figure 2. Chronic circadian misalignment decreases fly longevity



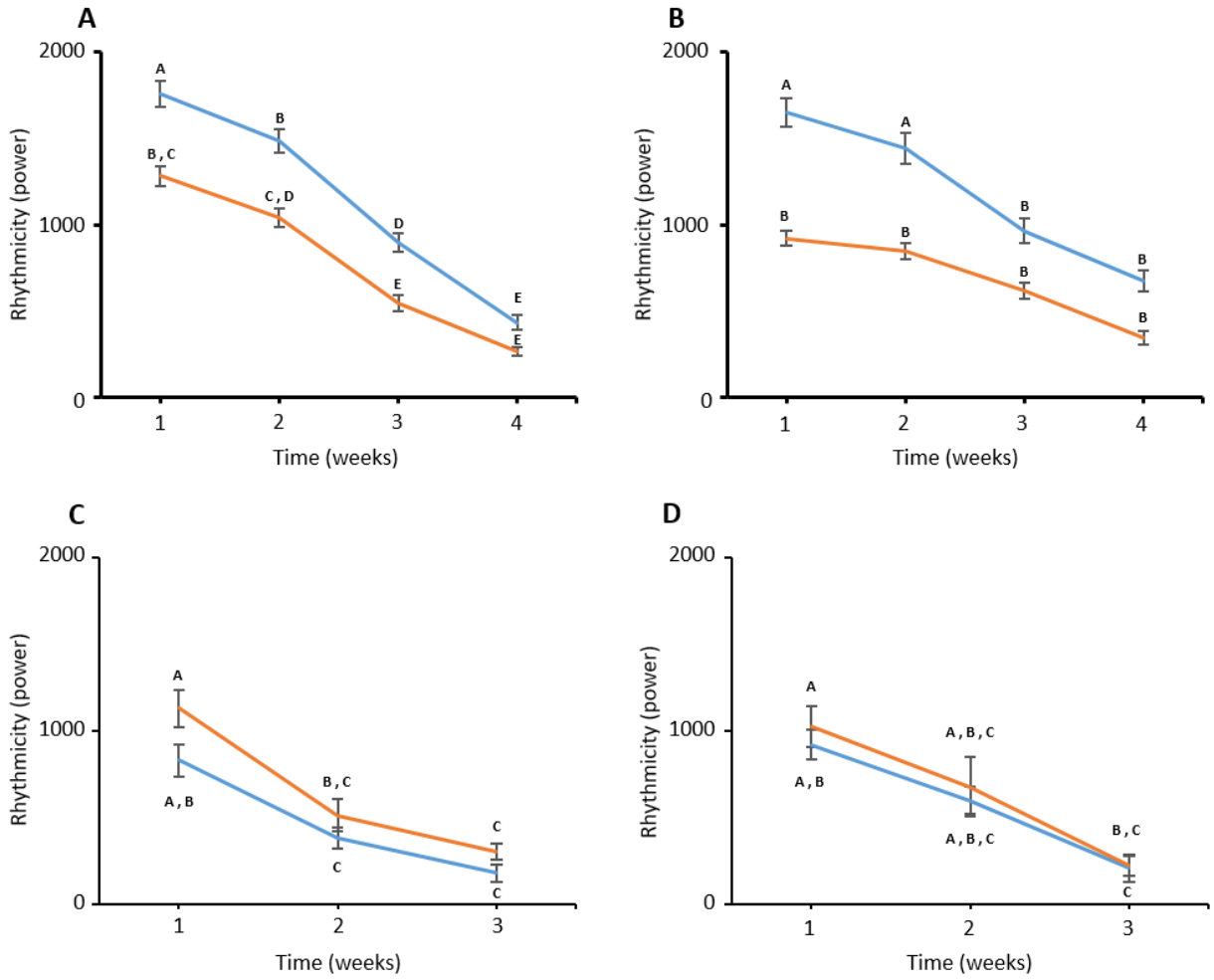
(A-B) Percent of flies surviving during exposure to either a control 24-hour day (blue line) or a chronic jetlag 28-hour day (orange line) throughout a 40-day period. Chronic jetlag results in decreased longevity for both female (A; 14.78% reduction, $n_{control}$=124, n_{jetlag}= 126, p=1.56e-04; LogRank test) and male (B; 14.72% reduction, $n_{control}$=125, n_{jetlag}= 120, p=2.65e-07; LogRank test) flies.

Figure 3. Locomotor activity behavior is altered by CCM



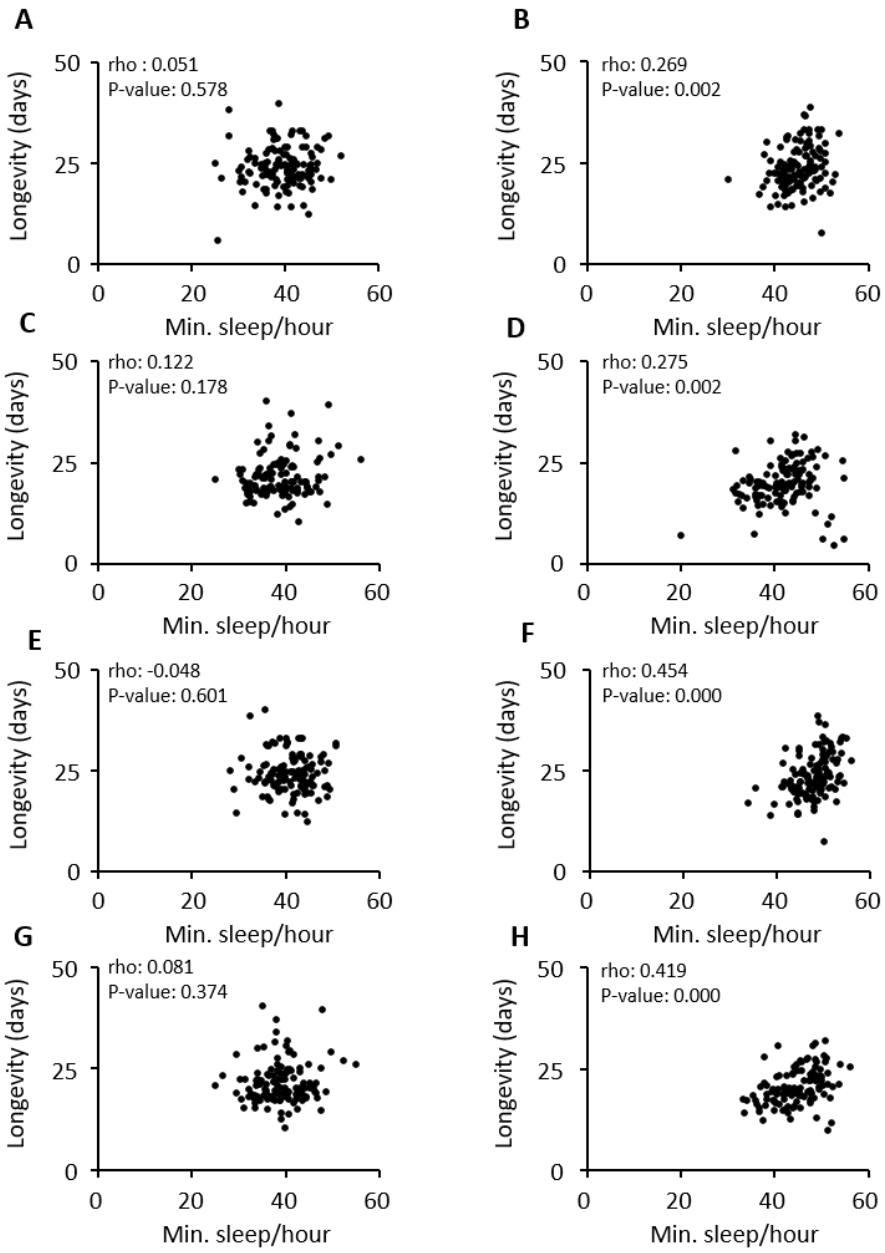
(A-H) Weekly average locomotor activity, containing mean number of beam breaks/min during 30-minute blocks of time. The white bars correspond to light periods and the black bars correspond to dark periods. Error bars represent +/- standard error measure. (A-D) Average activity during the first week of either control (A-B) or jetlag (C-D) schedules. Both male (C) and female (D) jetlag flies experienced early anticipation of activity to light transitions (indicated by red arrows). (E-H) Average locomotor activity during their third week of life in either the control (E-F) or jetlag (G-H) condition. Jetlag female (G) and male (H) flies maintain early anticipation behaviors while having a reduced activity bout at lights-off (indicated by blue arrows) compared to control females (E) and males (F).

Figure 4. Chronic circadian misalignment leads to a reduction in locomotor rhythmicity



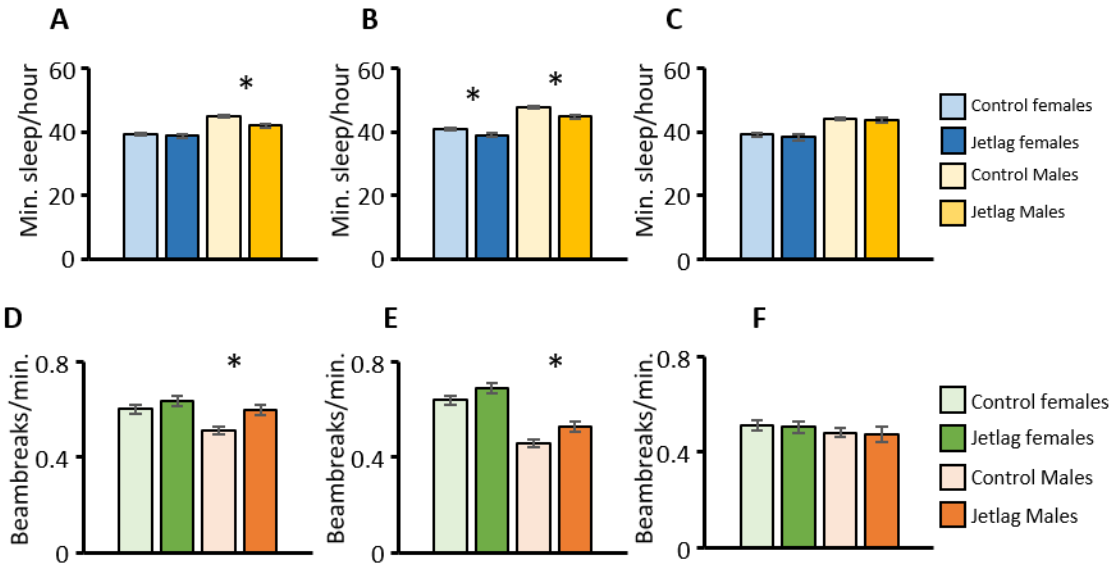
(A-B) Average locomotor activity rhythm strengths of flies exposed to either a control (24-hour) day (blue line) or a chronic jetlag (28-hour) day (orange line). Data points correspond to the average locomotor rhythm strength of each group during that week of the experiment. Different letters indicate data points that are statistically different than one another (Tukey's HSD test, $p<0.05$). Locomotor activity rhythmicity was reduced in jetlag flies for both females (A; $n_{control}=124$, $n_{jetlag}=126$, $p=0.000$; 2-way ANOVA; main effect of treatment) and males (B; n= $n_{control}=125$, $n_{jetlag}=120$, $p=0.000$; 2-way ANOVA; main effect of treatment) throughout the entire lifespan compared to control flies. (C-D) Average locomotor activity rhythm strengths of flies placed in DD following exposure to varying durations of either condition. Neither females (C; $n_{control}=14$, $n_{jetlag}=15$) nor males (D; $n_{control}=16$, $n_{jetlag}=15$) showed differences in rhythm strength when comparing week to week (Tukey's HSD test, $p<0.05$).

Figure 5. Baseline sleep duration is correlated with longevity in male flies



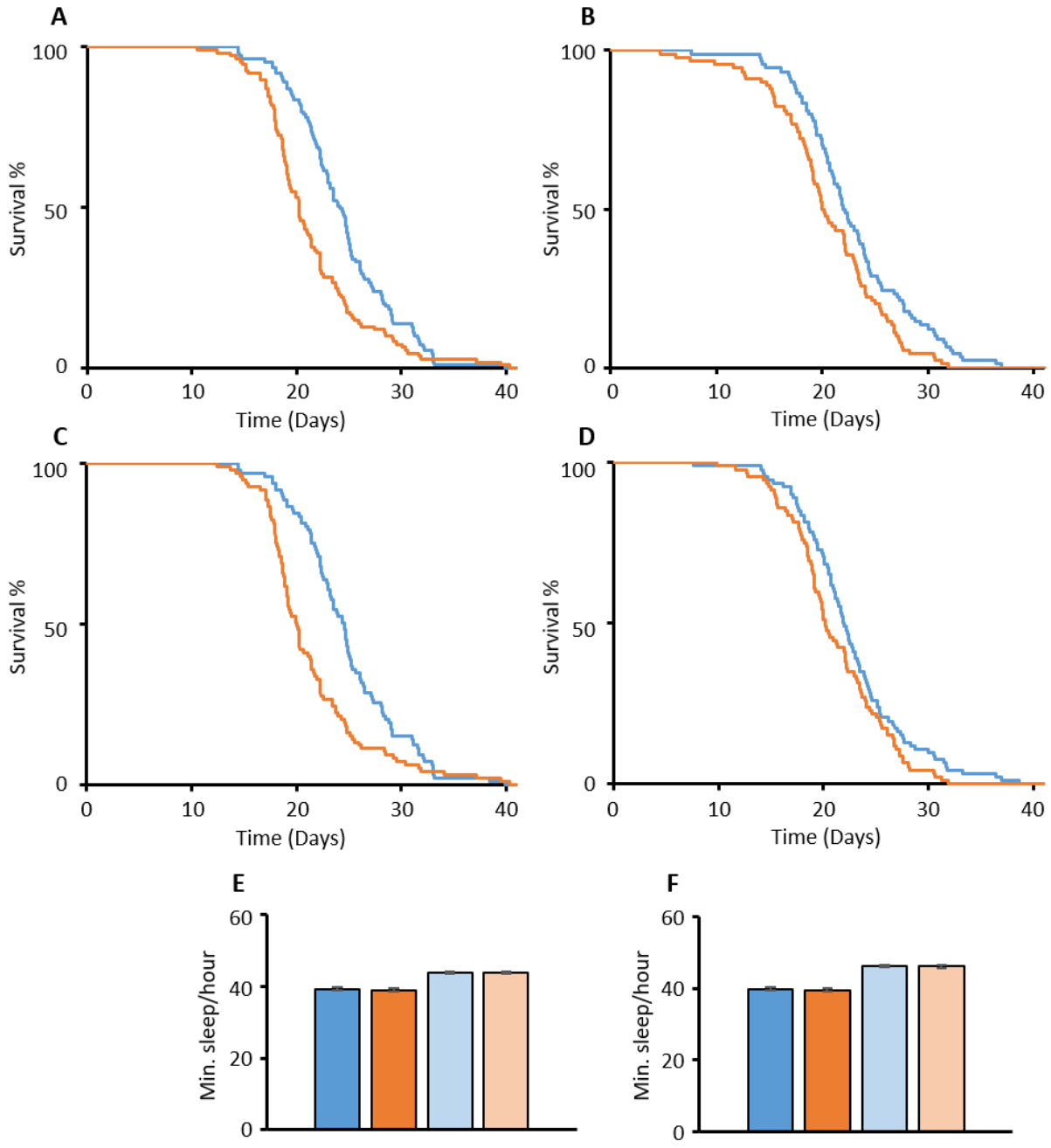
(A-H) Scatterplot of longevity against sleep. Each dot represents an individual fly. (A-D) average sleep/hour during the full lifetime of the fly is plotted against longevity for flies exposed to either control (A-B) or jetlag (C-D) conditions. While female control (A) and jetlag (C) showed no relationship, both control (B) and jetlag (D) males expressed significant, positive correlations ($p<0.05$; Spearman Rank). (E-H) Week 1 average sleep/hour against longevity for control (E-F) and jetlag (G-H) flies. Female control (E) and jetlag (G) flies expressed no correlation, while control (F) and jetlag (H) males did ($p<0.05$; Spearman Rank).

Figure 6. Sleep and activity amounts between treatments



(A-C) Sleep amounts (average min. sleep/hour) of control and jetlag flies for full life (A), week 1 (B), and week 3 (C). Males had differences in whole life (p=0.000, 2-tailed t-test) and week 1 sleep (p=0.000, 2-tailed t-test), while this wasn't maintained into week 3 (p= 0.616, 2-tailed t-test). Females had altered sleep during week 1 (p=0.002, 2-tailed t-test), while no changes were seen in whole life and week 3 (p >0.05, 2-tailed t-test). (D-F) Activity amounts (average beambreaks/min.) of control and jetlag flies for full life (D), week 1 (E), and week 3 (F). Similarly, males displayed whole life (p=0.002, 2-tailed t-test) and week 1 (p=0.013, 2-tailed t-test) changes in activity, which wasn't maintained into week 3 (p=0.863, 2-tailed t-test). Females had no differences in activity (p>0.05; 2-tailed t-test). *p<0.05.

Figure 7. Sleep matched flies maintain reduction in longevity



(A-D) longevities of flies matched for full life sleep (A-B) and week 1 sleep (C-D). Both females (A, $\chi^2 =$ 13.7, p=0.0002, LogRank; C, $\chi^2 = 14.3$, p=0.00016, LogRank) and males (B, $\chi^2 = 8.4$, p= 0.004, LogRank; D, $\chi^2 = 4.3$, p= 0.037, LogRank) maintained reductions in longevity. (E-F) Sleep amounts (min. sleep/hour) of flies included in longevity analysis. Sleep matching produced no significant differences in full life (E) and week 1 (F) sleep (p>0.05, 2-tailed t-test).

CHAPTER TWO

CIRCADIAN MISALIGNMENT INDUCES LARGESCALE CHANGES IN GENE EXPRESSION IN DROSOPHILA

Central Hypothesis: Chronic circadian misalignment leads to an overall reduction in health and well-being in *Drosophila melanogaster*.

Specific Aim 2: Investigate changes in gene transcription of *Drosophila melanogaster* exposed to a chronic jetlag schedule.

- Hypothesis: Exposing flies to a 28-hour (chronic jetlag) schedule will induce changes in gene transcription, specifically for genes involved in stress response pathways.
- Approach: Conduct whole-body RNA sequencing to assess changes in gene expression during exposure to a 28-hour (chronic jetlag) schedule. Conduct fluorescent microscopy with specific stress gene reporter lines to confirm these results.

Background

Epidemiological studies in humans have consistently demonstrated an association between CCM-inducing schedules and disease (Tokumaru et al, 2006; Stevens, 2009; Aspholm et al, 1999; Cho et al, 2000, Costa, 1996). To understand the connection between CCM and disease, researchers have used mammals to model and investigate the physiological consequences of CCM in controlled laboratory conditions (Golombek et al, 2013). While these

studies have confirmed many of the negative health effects of CCM in humans such as increased instance of cancer, obesity, and cognition deficits (Filipski et al, 2004; Fonken et al, 2010; Gibson et al, 2010), few have attempted to characterize the molecular mechanism behind such phenotypes. One recent study, however, assessed changes in gene transcription in the liver of mice following exposure to CCM, which was termed "chronic circadian rhythm disruption" (Van Dycke et al, 2015). In doing so, they identified changes in the transcription of specific genes, including CD36, which is a biomarker suggested to be indicative of metabolic syndrome and increased risk of human breast tumorigenesis (Handberg et al, 2006; Uray et al, 2004). These data indicate that the consequences of CCM may involve changes in overall gene expression.

Results from Chapter 1 indicated a reduced longevity independent of behavioral changes and a damaged core clock. Thus, our investigation shifted towards an assessment of changes occurring at the cell and molecular level. We hypothesized that by focusing on changes in gene expression in jetlagged flies, identifying specific genes and associated pathways would further explain the reduced longevity phenotype. This approach began by using RNA sequencing to compare gene expression levels of flies experiencing CCM (28-hour schedule) compared to a normal schedule (24-hour schedule). While flies exposed to 2 weeks only exhibited 7 genes displaying differential expression, the majority involved lipid metabolism. Interestingly, when we then assessed changes following 3 weeks exposure, we identified 351 genes displaying differential expression, including those involved in cellular stress and neuronal/synaptic function and maintenance.

Because results from RNA sequencing indicated an increased cellular stress response in flies exposed to CCM, we conducted fluorescent microscopy on transgenic reporter lines associated with various stress response pathways in an attempt to independently corroborate the

association between CCM and the stress response. This included the use of reporter lines in which green fluorescent protein (GFP) fluorescence should reflect the expression of the stress-related genes heat shock protein 22 (hsp22), hsp70, and glutathione S-transferase D (gstD), as well as an additional reporter line in which dsRed fluorescence should reflect the activation of the stress-related Jun Kinase pathway (TRE-dsred).

Both hsp22 (heat shock protein 22) and hsp70 (heat shock protein 70) genes have been found to be upregulated during normal aging, heat and oxidative stress, and hsp22-GFP and hsp70-GFP lines have been constructed which contain GFP downstream each of the genes promotor region (Yang and Tower, 2009). The TRE-dsred line involves the red fluorescent protein downstream of TRE (tetradecanoylphorbol acetate reponse element), which is activated in response to oxidative stress through Jun-N-terminal Kinase (JNK) signaling (Santabárbara-Ruiz et al, 2015; Chatterjee and Bohmann, 2012). Also involved in oxidative stress pathways, the gstD-GFP line contains an antioxidant response element (ARE) that is activated through Nrf2 signaling (Sykiotis and Bohmann, 2008). We reasoned that the use of these lines would allow us to assess whether chronic jetlag induced changes in expression of these genes and pathways in a manner similar to that observed following acute oxidative or heat stress, further confirming RNA sequencing data.

Our fluorescence microscopy involved the exposure of flies to either control (24-hour) or chronic jetlag (28-hour) conditions. In doing so, we generally found natural increases in fluorescence as flies aged, but no differences in reporter gene expression in flies exposed to CCM. This result in seemingly at odds with the upregulation of stress response genes observed in our RNA sequencing (which identified increased expression of both hsp22 and hsp70 following three weeks of exposure to CCM). One possibility is that changes in expression were occurring

in a tissue-specific manner which was undetected by our microscopy. Nevertheless, our results overall indicate large-scale changes in gene expression when flies are exposed to a CCM-inducing schedule, providing information about candidate molecular mechanisms leading to a reduction in longevity.

Methods

RNA Extraction

Iso31 flies were loaded into DAM monitors in control or chronic jet lag conditions. RNA extractions were done from ZT0-ZT3 after 2 or 3 weeks of control or jet lag exposure. Flies were anesthetized on CO_2, followed by the collection of 10 males and 10 females from each monitor in each condition into Eppendorf tubes on ice. We then added 200 uL TRI Reagent to each tube and homogenized with pestles. An additional 800 uL TRI Reagent was then added for a total of 1000 uL. To help with phase separation, 50 uL of 4-bromoanisole was added to each tube. The samples were then vortexed vigorously for 15 seconds. To produce a sample with greater purity, we centrifuged each sample for 15 minutes at 12,000 x g at 4^{o}C in cold centrifuge. Upon centrifuge completion, 500 uL of the aqueous phase was transferred to a new eppendorf tube. We then added 500 uL 100% ethanol and inverted tubes ~10x to thoroughly mix. Roughly 500 uL (half of the solution) was transferred to a Zymo-Spin IIC Column and centrifuged for 30 seconds at 16,000xg at RT. This step was repeated for the remaining 500 uL while discarding flow-through. We then treated samples with DNAse directly on the Zymo-Spin IIC column to remove any genomic DNA (according to the manufacturers instruction). Following DNAse treatment, we added 400 uL Direct-zol RNA PreWash. Tubes were centrifuged for another 30 seconds and this step was repeated. We then added 700 uL RNA Wash Buffer and centrifuged again for 1 minute at 16,000xg at RT. We discarded the flow through and centrifuged for another 2 minutes to

ensure the Wash Buffer was completely removed. To elute RNA, we added 60 uL DNase/RNase-Free Water directly to column matrix and centrifuged 30 seconds at 15,000xg at RT. OD readings for each individual sample were conducted to assess purity. Finally, we separated out 40 uL of each sample into new eppendorf tubes, which were stored at -80^{o}C and sent to Novogene for RNA sequencing.

RNA Sequencing

Library preparation and 150 base pair, paired-end RNA sequencing were conducted by Novogene (Davis, CA). >20 million reads were obtained per sample.

Differential Gene Expression Analysis

We conducted a differential gene expression analysis to determine whether specific genes displayed a significant difference in read counts (expression) between control and jetlag flies. We used RNA Star to map reads to the fly genome, mmquant to quantify number of reads mapped to each gene in each sample, and DEseq2 for differential expression analysis. Fold changes (FC) were expressed in log2 form, allowing positive and negative FC values to be equidistant to 0. Up- or downregulated genes were determined by DEseq2 with a false discovery rate (FDR) of 0.1. We did not filter genes based on fold change.

GO Term Analysis

We conducted Gene Ontology (GO) term analysis with the Princeton GO-term finder (https://go.princeton.edu/cgi-bin/GOTermFinder) to identify functional gene categories among biological processes that were over- or underrepresented among our differentially expressed genes. Analysis was done separately for up- and down-regulated genes from each week. The Bonferonni adjusted p-value cutoff was set to 0.05 to determine over- or underrepresented GO terms. The resulting lists were then passed through REVIGO (http://revigo.irb.hr) with an

allowed similarity of 0.7 to remove redundant terms. For tables, we further filtered the GO term list to remove highly generic GO terms. To do this, we determined the maximum distance to root term for each term and only included terms with a maximum distance of 4 or greater. Only 16 terms were included in these tables that held the lowest, most significant p-values.

Reporter Line Outcrossing

To control for the differences in genetic background, genetic outcrossing was conducted on the transgenic strains. These lines carry a *w* allele closely linked to their reporter transgene, which allowed for eye color to determine presence of our desired transgene after 8 successive outcrosses. Following the 8th outcross, virgin females were selected and crossed to either a *sco/cyo* or *TM2/TM6C,sb* balancer depending on the chromosomal location of the transgene.

Fluorescent Microscopy

Reporter flies were loaded into DAM monitors and placed in either control or chronic jetlag conditions. Following 1, 2, 3, or 4 weeks of exposure to either condition, males and females were removed from monitors and anesthetized using FlyNap for 60 seconds. Anesthetized flies were placed in a petri dish and positioned with the dorsal side facing up. No adhesive tape was used during the flies positioning. Flies were imaged under an Axiocam 503 mono microscope. Blue or green light was used to activate fluorescence, and images were taken and recorded under varying lighting exposures. These images were then analyzed using ImageJ, in which fluorescence was measured by pixel intensity in outlined abdomen, thorax, and heads of the flies.

Results

CCM induces largescale changes in gene expression

Our sleep matching analysis indicated that the reduction in longevity associated with CCM is independent of changes in behavior (including activity and sleep amounts). We therefore

hypothesized that the physiological consequences must be occurring due to molecular changes brought upon by CCM. To investigate this, we assessed levels of gene transcription by conducting whole fly RNA sequencing on combined male and female flies following both 2 and 3 weeks of jetlag or control exposure.

To assess overall changes in gene transcription between conditions, we conducted a differential expression analysis between control and jetlagged flies. By comparing control and jetlag groups following 2 weeks exposure, 7 genes were found to display differential expression (6 upregulated and 1 downregulated) (FDR <0.1) (**appdx. A**). While this list was small, the majority involved lipid metabolism. When we conducted the same analysis between control and jetlag flies at 3 weeks exposure, we found 351 genes exhibiting differential expression (245 downregulated and 106 upregulated) (adj. p <0.1) (**appdx. A)**. Some hallmark examples of upregulated genes involve the stress response, such as hsp22 and hsp70. The significant increase in the number of genes from week 2 to week 3 indicates prolonged exposure to CCM leads to greater molecular consequences.

While our differential expression analysis determined that CCM produced changes in gene expression, the mechanism leading to such changes in gene expression and whether they were maintained throughout the experiment remains unclear. To investigate this, we determined whether genes exhibiting differential expression at weeks 2 and 3 were correlated with their expression during the opposite week. For week 2, despite the fact that we didn't find a statistically significant correlation between the 7 genes that displayed differential expression and their fold change in expression at week 3 (adj. p=0.101, Pearson's Correlation), the data appeared to be trending toward a positive correlation (**fig.8A**). Furthermore, three of the six downregulated genes in week 2 were also determined to be significantly downregulated in week

3, while the remaining showed the same up- and downregulatory trends. This suggests that CCM may be producing immediate changes in gene expression that remain present throughout the remainder of the fly's life. Interestingly, when we assess fold change in expression of these same genes in control flies from weeks 2 to 3, 3 genes that were strongly downregulated in jetlagged flies at week 2 were actually upregulated in controls during aging (**fig. 8B**). This indicates that chronic jetlag may be effecting the fly's ability to modulate gene expression during natural aging.

Due to the large number of genes displaying differential expression at week 3, we conducted separate correlation analyses for those expressing up- and downregulation. When assessing differentially downregulated genes at week 3, we found no correlative relationship in expression of these genes during week 2 (adj. p=0.481, Pearson's Correlation) (**fig. 10A**). However, when we conducted this same analysis in differentially upregulated genes, we identified a positive correlation (adj. p=0.002, Pearson's Correlation; **fig. 10B**). This demonstrates that the specific genes that are strongly upregulated following three weeks of jetlag exposure are already showing signs of upregulation by week 2, though these changes are not statistically significant at the 2-week time point. Furthermore, when comparing differentially up- and downregulated genes in week 3 jetlagged flies to their fold change in expression among normal aged flies, several genes were found to exhibit an opposite effect following exposure to jetlag compared to natural aging. (**fig. 10C, D**). We identified 11 genes that were upregulated by CCM that exhibited a natural downregulation in expression in control aged flies (**fig. 10D**). Similarly, 22 genes that were differentially downregulated in week 3 jetlagged flies displayed an upregulation in control aged flies (**fig. 10C**). This, along with our week 2 data, determines that CCM produces adverse changes in gene expression that are opposite, in some cases, to those that

occur during a flies natural aging process. Further investigation of these genes may lead to a deeper understanding of the mechanisms behind the CCM-induced reduction in longevity.

Go term analysis displays reoccurring themes of gene expression consistent with reduced longevity

To determine whether the differentially expressed genes were enriched for specific functional categories, we conducted a GO term analysis. This allow for a more rationalized and simplified version of which processes and pathways were being effected following exposure to chronic jetlag. Despite only identifying 7 genes displaying differential expression at week 2, GO term analysis on the 6 downregulated genes identified 4 GO terms that mainly involved the fly's lipid metabolism. When this analysis was then conducted on week 3 genes, we identified 18 GO terms associated with week 3 upregulated genes and 178 GO terms associated with week 3 downregulated genes (**appdx. B).**

Table 1 shows the top 16 most statistically significant GO terms among downregulated genes during week 3. This list revealed several GO terms that may explain the reduced longevity in jetlagged flies, including "Regulation of Gene Expression, "Cell Development", and "System Development." We also noticed that 2 of the 16 involved the nervous system. This included "Nervous System Development" and "Neurogenesis" (**table 1).** When we then assessed all overrepresented GO terms (appendix B), we identified 10 additional terms related to the nervous system (**table 3**), thus suggesting some level of neurological damage in jetlagged flies.

Table 3 shows the top 16 most statistically significant GO terms among upregulated genes. Interestingly, many of these terms are involved in cellular stress response, including "Response to Oxidative Stress", "Response to Hypoxia", and "Response to Unfolded Proteins". Genes that fell into these categories included hsp70 and hsp22. These data suggest that chronic

jetlag may be inducing some level of endogenous stress, potentially leading to a damaged nervous system.

Use of reporter lines to assess physiological consequences of CCM

To further assess changes in gene expression, and to potentially confirm results from RNA sequencing, we determined the effect of CCM on the expression of specific transgenic reporter lines by conducting whole-fly fluorescence microscopy. These reporter lines included the following: hsp22-GFP, hsp70-GFP, gstD-GFP, and TRE-dsred. We began by initially testing the functionality and responsiveness of these lines to normal stressors, and found that all lines exhibited increased whole-body fluorescence following exposure to acute heat stress (**figure 8A-C**). Due to these lines' responsiveness to such stress, we reasoned that we could use them to determine whether some level of endogenous stress was occurring during CCM.

We assayed for reporter gene expression following varying amounts of time in either a 28-hour (jetlag) or a 24-hour (control) schedule. We generally found gradual increases in fluorescence as flies aged in both groups, consistent with previous work indicating age-associated increases in cellular stress (Yang and Tower, 2009). However, CCM didn't appear to produce changes in expression in any of our stress gene reporter lines. The first line we assayed was hsp22-GFP, in which female and males showed increased fluorescence intensity as flies aged (females, $F_{(3, 92)}$ =1448.05, p=0.000; males, $F_{(3, 106)}$ =947.44, p=0.000, 2-way ANOVA; main effect of week; **fig. 8D-E**). While females did show a main effect of treatment ($F_{(1, 92)}$ =13.38, p=0.000, 2-way ANOVA), jetlag flies only displayed a significant elevation in fluorescence at week 4 (Tukey's HSD, p=0.000, **fig. 8E**). Furthermore, a main effect of treatment was not seen in the males ($F_{(1, 106)}$ =0.00, p=0.954, 2-way ANOVA), overall indicating limited changes in fluorescence between control and jetlag conditions among males and females.

We conducted the same experiment on hsp70 flies. Surprisingly, both sexes lacked natural increases in fluorescence as they aged, despite the fact that these flies have previously been reported to undergo age-associated increases in reporter gene expression (Yang and Tower, 2009). Furthermore, this line lacked a significant effect of treatment (females, $F_{(1,94)}$ =2.34, p=0.13; males, $F_{(1, 84)}$ =0.13, p=0.722, 2-way ANOVA, main effect of treatment), as well as a lack of difference in fluorescence between control and jetlag groups at each week of exposure for both sexes (Tukey's HSD p>0.05; **fig. 8F-G**). Thus, these results failed to confirm data from RNA sequencing, in which hsp70 and hsp22 expressed a significant upregulation in CCM conditions, specifically at 3 weeks exposure (**appdx. A**). This could potentially result from tissue specific changes in hsp70 and hsp22 expression, which would not be detected by our whole-fly fluorescence imaging.

Results for both the TRE-dsred and gstD-GFP lines demonstrated that both sexes underwent natural increases in fluorescence as flies aged (female TRE-dsred, $F_{(3, 106)}$ = 54.95, p<0.05; female gstD-GFP, $F_{(3, 118)}$ = 147.65, p<0.05; male TRE-dsred, $F_{(3, 95)}$ = 44.19, p<0.05; male gstD-GFP, $F_{(2, 74)}$ = 70.15, p<0.05, 2-way ANOVA; main effect of week). However, as was the case for the hsp lines, we observed no differences in fluorescence when comparing the different treatments each week (Tukey's HSD p>0.05; **fig. 8H-K**). It should be noted that we only analyzed 3 weeks of data for male gstD-GFP flies due to mortality.

Overall, these data indicate that while expression of these stress genes and pathways increased as flies aged, the effect of CCM did not lead to a greater level of gene expression. These data, especially for hsp70 and hsp22, were unexpected when considering results from RNA sequencing. This may be due to the limitations of our fluorescence analysis, in which we are observing expression changes in the whole body (abdomen and thorax), instead of using a

tissue-specific approach that may indicate such changes elsewhere. On the other hand, our RNA sequencing results may have partial inaccuracy due to false positives generated when determining genes expression changes at the level of significance. However, this is unlikely given that RNA sequencing showed the upregulation of a number of genes associated with stress responses.

Discussion and Conclusions

Results from chapter 1 ruled out several possibilities that could have produced the reduction in longevity produced by CCM, including damage to molecular cycling and changes in locomotor activity and sleep amounts. To continue this investigation, we chose to investigate changes occurring at the cellular and molecular level, which included the assessment of gene expression in flies exposed to CCM. In this chapter, we report several important findings that bring us one step closer to fully characterizing the mechanisms behind reduced longevity and physiological health following exposure to aberrant lighting schedules.

Firstly, we indicate largescale changes in gene transcription in flies exposed to prolonged exposure to a 28-hour (chronic jetlag) schedule. While only 7 genes exhibited differential expression following 2 weeks of CCM, this increased to 351 differentially expressed genes after 3 weeks, thus indicating a larger effect following a longer period of misalignment. These are consistent with epidemiological studies, in which those exposed to years of chronic jetlag are more likely to develop cancer and cardiovascular disease (Tokumaru et al, 2006; Stevens, 2009; Costa, 1996). Furthermore, it suggests those working in CCM-inducing occupations are more prone to physiological changes compared to infrequent transmeridian travel and lighting aberrations. Repeating this experiment in mammals that have a greater median lifespan would

allow us to assess RNA expression at more time intervals, potentially producing more evidence to support prolonged exposure to CCM results in greater gene expression changes.

We then chose to conduct correlation analyses between genes exhibiting differential expression and their fold changes in expression during the opposite week of the experiment. This showed that despite a lack of statistical significance (potentially due to low N; p=0.101, Peason's correlation), a scatterplot of the gene expression for the 7 genes exhibiting differential expression in week 2 appeared to be trending toward a positive correlation with their fold change in expression during week 3 (**fig. 8A**). These data suggest that chronic jetlag and CCM may be producing immediate molecular consequences, which are maintained into prolonged exposure. When we compared fold change of these differentially expressed genes with their fold changes during normal aging, we determined 3 genes to have the opposite effect in expression, indicating that the genetic changes induced by jetlag are not simply caused by an advanced aging process.

We found that many more genes were significantly differentially expressed following 3 weeks of jetlag compared to only 2 weeks. However, subsequent analysis demonstrated a positive correlation between fold change in week 3 compared to week 2 for those genes that were upregulated by jetlag. Thus, many of these genes were already trending towards increased expression in week 2. What this showed was that a gene that expresses CCM-induced upregulation during later life exhibited signs of these changes during early exposure to misalignment. When we then compared up- and downregulated genes at week 3 to their fold change in expression during normal aging, we determined several genes that exhibited the opposite effect in expression (**fig. 9C, D**). This tells us that CCM may be inhibiting or disrupting later life changes in gene expression, potentially contributing to the fly's reduced longevity (**fig. 2**).

We also conducted a GO term analysis to understand the changes in gene expression in a categorical manor and identify functional gene categories that are overrepresented among our differentially expressed genes. In doing so, we determined a large portion of these genes are involved in general biological processes, indicating a broad consequence of CCM. Regarding week 2 differentially downregulated genes, we found that despite only 4 GO terms generated, the majority involved lipid metabolism. When considering previous work reporting evidence to suggest a relationship between fly lipid metabolism and aging (Hansen et al, 2013), it is possible that early changes in fly lipid metabolism brought upon by CCM may be leading to its reduced longevity. When we then observed differentially upregulated genes during week 3, we found several overrepresented GO terms involved in cellular stress, which has previously been shown to produce a reduction in lifespan (Fleming et al, 1992). Finally, when assessing downregulated genes, we identified overrepresented GO terms associated with the nervous system, thus suggesting some level of damage to be occurring. Collectively, these findings suggest that CCM may be producing early changes in lipid metabolism, causing an increase in cellular stress, further leading to an effected or damaged nervous system that could explain reduced longevity in jetlagged flies. Furthermore, because the endogenous clock functions through the nervous system, this could be predictive of aberrations of other behaviors dictated by the circadian timing system when experiencing CCM. Additional analyses could involve the assessment of flies mating behaviors following exposure to our 28-hour (chronic jetlag) schedule.

As a method to confirm our results from RNA sequencing, we chose to conduct whole-body fluorescence microscopy on flies as a visual indication of gene expression. In doing so, we selected 4 transgenic lines (hsp22-GFP, hsp70-GFP, gstD-GFP, TRE-dsred) that were known to be involved in both heat and oxidative stress. Surprisingly, we found no significant changes in

expression of these lines when exposed to CCM. Because gstD and the jun kinase pathway had not be implicated by our RNA sequencing, these results did not come to a surprise. Furthermore, these lines represent a small fraction of pathways involved in the flies stress response. Today, several more transgenic reporter lines are available involving other genes associated with the fruit flies stress response. One of which includes STAT-GFP, which is activated through JAK/STAT signaling involved in the flies' immune response (Zeidler and Bausek, 2013). The use of these going forward may determine whether the changes in a fly's stress response is among the consequences of CCM.

Genes reported in RNA sequencing to be upregulated significantly were hsp70 and hsp22, specifically at week 3 (**appdx. A**). Despite these results, neither hsp22 and hsp70 reporter flies showed a jetlag-related increase in expression. Furthermore, hsp70-GFP line failed to show an age-related increase in fluorescence as well. The absence of increased GFP fluorescence with age contradicts both our RNA sequencing results, which showed strong upregulation of hsp70 in control flies when comparing weeks 2 and 3, and also previous studies that observed increased hsp70-GFP expression with age (Tower, 2011). This absence in aged flies may be due to mortality during later weeks (weeks 3 and 4), such that only relatively healthy and unaffected flies are left for imaging. The lack of increased fluorescence among jetlagged flies in both hsp70 and hsp22 may be the result of either two possibilities. Firstly, upregulation may be occurring in a tissue specific manor that is undetected through our fluorescence microscopy. When considering the brain as the location of core clock neurons directly influenced by the 28-hour day (Allada and Chung, 2010), one would suspect changes in transcription centered to that area. Secondly, hsp22 and hsp70 may have been found significant due to false positives generated

during RNA sequencing. To investigate either theory, we must repeat fluorescence microscopy to increase N, following by a tissue-specific approach.

Future Directions

Data presented in chapter 1, including aberrant locomotor activity behaviors, suggested the 28-hour (jetlag) schedule was producing misalignment between endogenous and environmental clocks. To confirm misalignment, it would be wise to assess oscillation of specific clock genes including *per* and *tim* in fly brains. Building upon this, assessing oscillation of these proteins in peripheral tissues may indicate a second level of misalignment between core clock neurons and peripheral neurons.

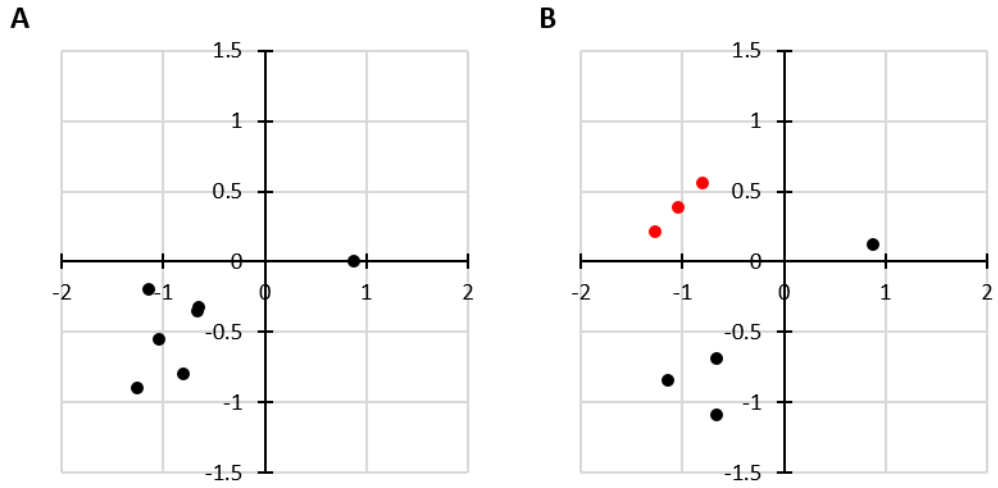
Due to the results from fluorescent microscopy failing to confirm RNA sequencing data, we would also like to conduct a tissue specific investigation going forward. Despite negative results form gstD-GFP and TRE-dsred, oxidative stress may be occurring directly in the brain, being the location of the central clock and light-input pathways (Allada and Chung, 2010). This can be assessed using a red mitochondrial superoxide indicator (MitoSox), which fluorescently labels areas of increased oxidative stress (Muliyil and Narasimha, 2014). Results would confirm RNA sequencing data and further suggest that the brain is the main cite of molecular consequences when experiencing CCM.

This tissue specific approach involving the brain may also include the investigation of specific genes that were either up or downregulated following exposure to CCM. One example includes brp, which codes for a specific bruchpilot (BRP) protein. Because of BRPs vital role in maintaining presynaptic active zones (AZs) (Kittel et al, 2006), its apparent downregulation in RNA sequencing data suggests neuronal/synaptic loss (**appdx. A**), potentially explaining reduced fly longevity. Using a monoclonal brp antibody, nc82 (Wagh et al, 2006), we can

determine whether levels of BRP are in fact reduced, further confirming RNA sequencing data and a damaged CNS.

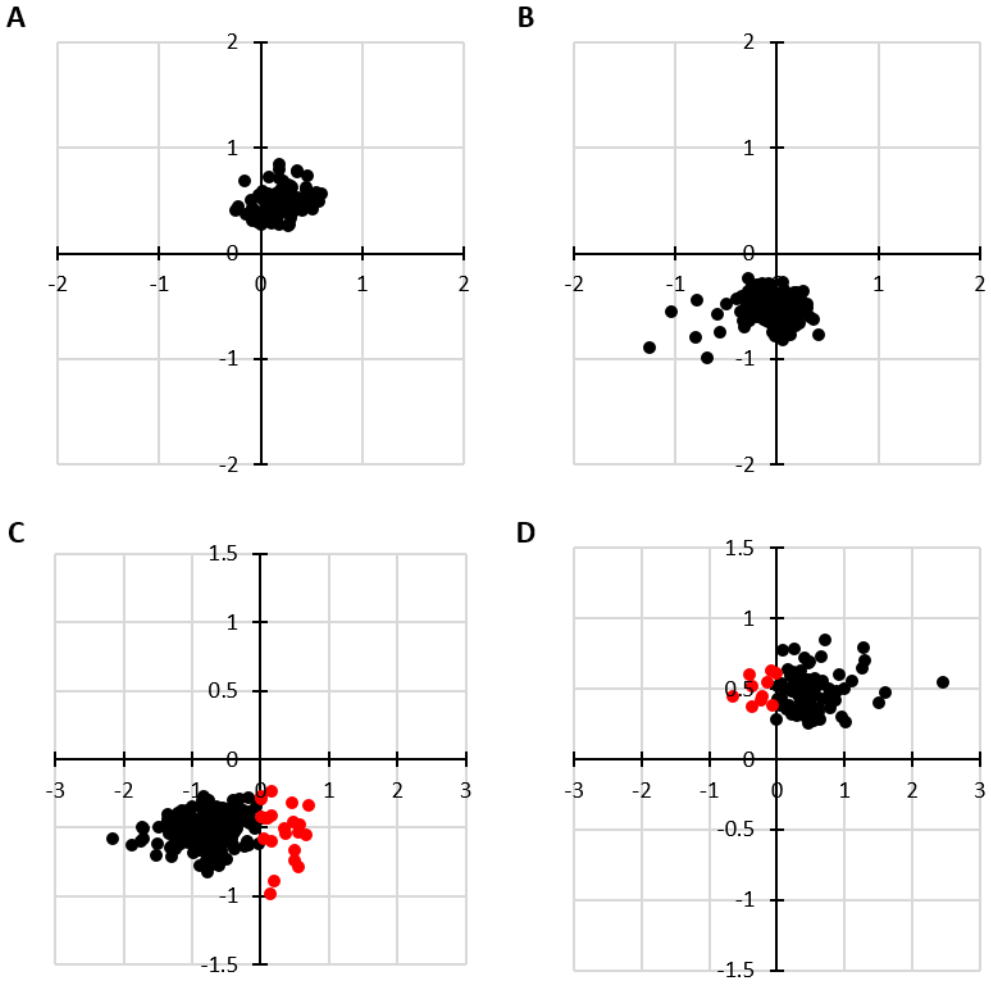
Figures

Figure 8. Differential expression correlation analysis for week 2 differentially expressed genes.



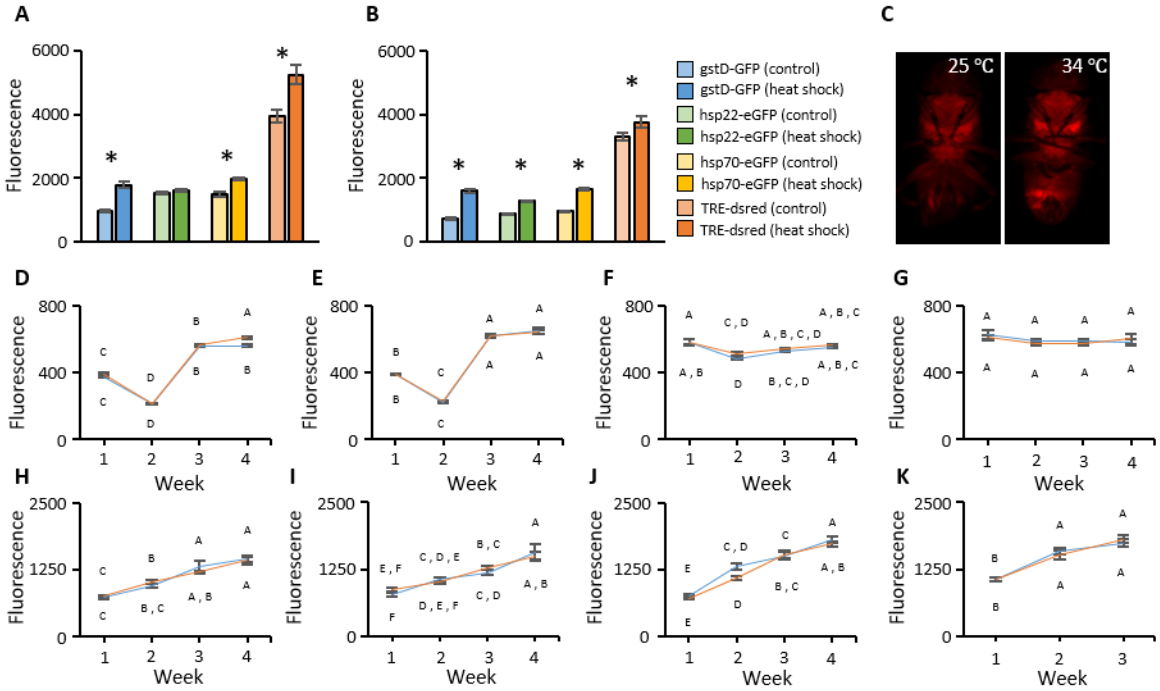
(A-B) Scatterplot of log2 fold change in gene expression between different groups, in which each dot represents a specific gene. (A) Week 2 jetlag induced log2 fold change (x-axis) of differentially expressed genes plotted against the log2 fold change for the same gene at week 3 (y-axis; adj. p=0.101, Pearson's Correlation). (B) Week 2 jetlag-induced log2 fold change (x-axis) plotted against its $\log_2$ fold change in aged control flies. Red dots refer to genes that exhibited opposite regulation in jetlag (downregulation) vs aging (upregulation). Note that not all fold changes in figure 8 were statistically significant.

Figure 9. Differential expression correlation analysis for week 3 differentially expressed genes.



(A-D) Scatterplot of log2 fold changes in expression among genes between different groups, in which each dot represents a specific gene. (A-B) Week 3 jetlag-induced log2 fold changes in genes exhibiting differential upregulation (A, 106 genes; adj. $p<0.1$) and downregulation (B, 245 genes; adj. $p<0.1$) (y-axis) plotted against their jetlag-induced log2 fold changes during week 2 (x-axis). While week 3 downregulated genes showed no correlative relationship (adj. $p=0.481$, Pearson's Correaltion), week 3 upregulated genes displayed a positive correlation with their fold changes in week 2 (adj. $p=0.002$, Pearson's correlation). (C-D) Week 3 jetlag-induced log2 fold change in expression plotted against the aging-induced log2 fold changes in control flies for the same genes. Red dots refer to genes that exhibited opposite regulation in jetlag vs aging. Note that not all fold changes in figure 10 were statistically significant.

Figure 10. Exposure to CCM fails to produce changes in whole-body stress gene reporter line expression



(A-B) Mean whole-body fluorescence for female (A) and male (B) reporter flies, indicating an increase in fluorescence when exposed to 34°C heat shock (*p<0.05, 2-tailed t-test). (C) Fluorescent image of TRE-dsred line representative of flies following 24-hour exposure to either 25°C or 34°C conditions. (D-K) Mean whole-body fluorescence of reporter lines exposed to either 24-hour (control; blue line) or 28-hour (jetlag; orange line) schedules for 1-4 weeks. (D) hsp22-eGFP females (n; control=13, jetlag=11), hsp22-GFP males (n; control=16, jetlag=10), (F) hsp70-GFP females (n; control=14, jetlag=16), (G) hsp70-GFP males (n; control=14, jetlag=16), (H) TRE-dsred females (n; control=15, jetlag=14), (I) TRE-dsred males (n; control=14, jetlag=14), (J) gstD-GFP females (n; control=16, jetlag=14), (K) gstD-GFP males (n; control=16, jetlag=16). Different letters indicate data points that are statistically different from one another (p<0.05, Tukey's HSD test).

Tables

Table 1. Overrepresented GO terms among downregulated genes.

Downregulated GO Terms						
GO_ID	TERM	CLUSTER FREQUENCY	GENOME FREQUENCY	CORRECTED_PVALUE	FDR_RATE	GENE NAME
GO:0048468	cell development	97 out of 240 genes, 40.4%	1397 out of 13900, 10.1%	1.02E-32	0.00%	Parp,dlp,mask,p120ctn,Fs(2)Ket,Not1,nudE,Ptp69D,norpA,Sxl,psq,stai,Galphao,Syx1A,Evl5,pod1,tud,zip,Ten-a,Myc,Dl,CG41099,Rbp6,mei-P26,chif,poe,fax,Appl,shg,dally,Pka-C1,kug,Syp,rin,eIF4G2,nrm,RhoGAP100F,wisp,pum,ftz-f1,pAbp,brat,msi,Trf2,sdk,dnc,cta,Rap1,kis,hth,lid,RhoGAPp190,Ephrin,PlexB,Rim,AGO1,shep,Top1,shn,osk,Inx2,Wnk,PlexA,Mical,nej,sif,dom,Pka-R2,zfh1,Tao,bun,ed,dlg1,eIF4G1,Cam,shot,Hrb27C,clu,sog,sd,spoon,pigs,rg,gish,orb,trx,retn,Rbfox1,Syt1,hdc,Bsg,sqd,Hrb98DE,bru1,bt,Fmr1,unk,ct
GO:0030154	cell differentiation	106 out of 240 genes, 44.2%	1688 out of 13900, 12.1%	1.13E-32	0.00%	Parp,dlp,skd,mask,p120ctn,Fs(2)Ket,Not1,nudE,Ptp69D,norpA,Sxl,psq,stai,Galphao,Syx1A,Evl5,Gbeta13F,pod1,tud,zip,Ten-a,Myc,Dl,cno,CG41099,Rbp6,rost,mei-P26,chif,poe,fax,Appl,shg,dally,Pka-C1,kug,Syp,rin,eIF4G2,nrm,RhoGAP100F,wisp,pum,ftz-f1,pAbp,brat,CtBP,msi,Trf2,sdk,dnc,cta,Rap1,kis,hth,lid,RhoGAPp190,Ephrin,PlexB,Rim,AGO1,shep,Top1,shn,osk,Inx2,Wnk,PlexA,Mical,nej,sif,dom,Pka-R2,zfh1,Tao,bun,ed,dlg1,eIF4G1,Cam,shot,Hrb27C,clu,sog,sd,spoon,pigs,rg,gish,orb,trx,retn,E(bx),kirre,Rbfox1,Syt1,hdc,Bsg,sqd,Hrb98DE,bru1,bt,Fmr1,pyd,unk,ct
GO:0007275	multicellular organism development	123 out of 240 genes, 51.3%	2346 out of 13900, 16.9%	1.02E-31	0.00%	Parp,dlp,skd,mask,p120ctn,nudE,Ptp69D,dpy,norpA,Sxl,eIF4EHP,Nckx30C,psq,stai,Galphao,Syx1A,Set2,CG14073,Gbeta13F,pod1,Su(Tpl),tud,zip,Ten-a,indy,Myc,Dl,cno,CG41099,chif,Pcl,poe,Plp,fax,Appl,shg,Sin3A,dally,wdb,Pka-C1,kug,Syp,rin,MYPT-75D,nrm,RhoGAP100F,crp,pum,ftz-f1,pAbp,brat,CtBP,msi,Trf2,gw,sdk,dnc,cta,Rap1,lolal,tim,kis,hth,lid,CG43658,puf,RhoGAPp190,Ephrin,PlexB,Rim,Reph,AGO1,shep,Top1,shn,osk,Inx2,Wnk,PlexA,Mical,nej,sif,nmo,chrb,dom,Pka-R2,zfh1,Tao,bun,ed,dlg1,rdx,Cam,shot,mgl,Cp190,Hrb27C,clu,akirin,CaMKII,sog,scl,spoon,rg,gish,scyl,orb,trx,retn,E(bx),kirre,Rbfox1,Syt1,hdc,Bsg,Ssdp,sqd,Hrb98DE,Fmr1,pyd,tou,unk,ct
GO:0009653	anatomical structure morphogenesis	98 out of 240 genes, 40.8%	1512 out of 13900, 10.9%	1.29E-30	0.00%	Parp,dlp,skd,mask,p120ctn,Fs(2)Ket,nudE,Ptp69D,dpy,norpA,psq,Arpc2,Galphao,Syx1A,Gbeta13F,pod1,zip,Ten-a,Myc,Dl,cno,CG41099,rost,chif,poe,Plp,fax,Appl,shg,dally,Pka-C1,kug,Syp,rin,MYPT-75D,RhoGAP100F,pum,ftz-f1,pAbp,brat,CtBP,Trf2,Ckialpha,sdk,dnc,cta,Rap1,lolal,kis,hth,CG43658,RhoGAPp190,Ephrin,PlexB,AGO1,shn,osk,Inx2,Wnk,PlexA,Mical,nej,sif,nmo,chrb,dom,Pka-R2,zfh1,bun,ed,dlg1,rdx,Cam,shot,Hrb27C,Slik,sog,sd,spoon,pigs,rg,gish,scyl,orb,trx,retn,kirre,Rbfox1,hdc,Bsg,sqd,Hrb98DE,bru1,bt,Fmr1,pyd,unk,ct
GO:0048731	system development	102 out of 240 genes, 42.5%	1722 out of 13900, 12.4%	7.36E-29	0.00%	dlp,skd,mask,p120ctn,nudE,Ptp69D,dpy,norpA,Sxl,Nckx30C,psq,stai,Galphao,Syx1A,Set2,CG14073,Gbeta13F,pod1,Su(Tpl),zip,Ten-a,Myc,Dl,cno,chif,Pcl,poe,fax,Appl,shg,Sin3A,dally,Pka-C1,kug,Syp,rin,MYPT-75D,nrm,RhoGAP100F,crp,pum,ftz-f1,pAbp,brat,CtBP,msi,Trf2,sdk,dnc,Rap1,lolal,kis,hth,lid,CG43658,puf,RhoGAPp190,Ephrin,PlexB,Rim,Reph,AGO1,shep,shn,Inx2,Wnk,PlexA,Mical,nej,sif,nmo,dom,Pka-R2,zfh1,Tao,bun,ed,dlg1,rdx,Cam,shot,Hrb27C,clu,akirin,CaMKII,sog,sd,rg,gish,trx,retn,E(bx),kirre,Rbfox1,hdc,Bsg,Hrb98DE,Fmr1,pyd,tou,unk,ct
GO:0048513	animal organ development	76 out of 240 genes, 31.7%	1197 out of 13900, 8.6%	1.39E-21	0.00%	dlp,skd,mask,p120ctn,dpy,norpA,Sxl,Nckx30C,psq,Galphao,Syx1A,Set2,CG14073,Gbeta13F,Su(Tpl),zip,Ten-a,Myc,Dl,cno,Pcl,Appl,shg,Sin3A,dally,Pka-C1,kug,rin,MYPT-75D,RhoGAP100F,crp,ftz-f1,pAbp,brat,CtBP,msi,Trf2,sdk,Rap1,lolal,hth,lid,kis,CG43658,RhoGAPp190,puf,Ephrin,shn,Wnk,nej,nmo,dom,zfh1,Tao,bun,ed,dlg1,Cam,rdx,shot,akirin,sog,gish,rg,sd,trx,retn,E(bx),kirre,Rbfox1,hdc,Hrb98DE,Fmr1,pyd,unk,ct
GO:0007399	nervous system development	72 out of 240 genes, 30.0%	1082 out of 13900, 7.8%	2.15E-21	0.00%	dlp,mask,p120ctn,nudE,Ptp69D,stai,Galphao,Gbeta13F,pod1,zip,Ten-a,Myc,Dl,cno,chif,Pcl,poe,fax,Appl,shg,Sin3A,dally,Pka-C1,Syp,rin,nrm,RhoGAP100F,pum,ftz-f1,pAbp,brat,sdk,dnc,hth,kis,RhoGAPp190,PlexB,Ephrin,Rim,Reph,shep,AGO1,shn,Wnk,PlexA,nej,Mical,nmo,sif,dom,Pka-R2,zfh1,Tao,bun,ed,dlg1,Cam,shot,Hrb27C,CaMKII,clu,gish,rg,trx,retn,Rbfox1,hdc,Bsg,Fmr1,tou,unk,ct
GO:0010468	regulation of gene expression	84 out of 240 genes, 35.0%	1492 out of 13900, 10.7%	7.02E-21	0.00%	Parp,Edc3,skd,Not1,dpy,Sxl,eIF4EHP,psq,mub,Atf6,Set2,AGO3,Su(Tpl),Pdp1,Myc,srl,Pcl,scrt,Sin3A,dally,Pka-C1,Syp,rin,Usp7,crp,CG2926,pum,ftz-f1,mamo,pAbp,brat,Su(z)2,CtBP,msi,Trf2,gw,Tis11,lolal,tim,hth,lid,kis,CG11486,puf,AGO1,shn,osk,Tob,MED26,nej,nmo,upSET,dom,zfh1,bun,dlg1,eIF4G1,Ncoa6,Cp190,Hrb27C,gpp,akirin,CG16779,sog,sd,DCP2,orb,fne,trx,Mnt,retn,E(bx),CG32767,Rbfox1,hdc,Nup153,Ssdp,sqd,Hrb98DE,bru1,Fmr1,CG4612,tou,ct
GO:0009888	tissue development	69 out of 240 genes, 28.8%	1047 out of 13900, 7.5%	4.72E-20	0.00%	Parp,dlp,skd,Fs(2)Ket,Not1,nudE,dpy,Sxl,psq,stai,Galphao,Syx1A,Set2,Evl5,CG14073,Gbeta13F,Su(Tpl),zip,Myc,Dl,cno,CG41099,chif,shg,Sin3A,dally,Pka-C1,kug,Syp,rin,MYPT-75D,ftz-f1,CtBP,cta,Rap1,hth,kis,CG43658,puf,shn,Inx2,Wnk,nej,nmo,dom,zfh1,Tao,bun,ed,dlg1,rdx,shot,Hrb27C,gish,pigs,spoon,sd,sog,orb,trx,Rbfox1,kirre,hdc,sqd,Fmr1,pyd,unk,ct
GO:0032989	cellular component morphogenesis	55 out of 240 genes, 22.9%	668 out of 13900, 4.8%	1.11E-19	0.00%	kis,dlp,RhoGAPp190,Ephrin,PlexB,mask,p120ctn,nudE,Ptp69D,shn,Arpc2,Wnk,PlexA,Mical,sif,dom,Pka-R2,zfh1,pod1,bun,ed,zip,Ten-a,Dl,cno,Cam,CG41099,chif,shot,fax,Hrb27C,Appl,shg,Slik,dally,spoon,pigs,Syp,trx,retn,RhoGAP100F,pum,ftz-f1,hdc,Bsg,brat,Ckialpha,dnc,bru1,bt,Fmr1,pyd,cta,Rap1,ct
GO:0022008	neurogenesis	62 out of 240 genes, 25.8%	862 out of 13900, 6.2%	1.51E-19	0.00%	kis,hth,dlp,RhoGAPp190,Ephrin,PlexB,mask,p120ctn,Rim,shep,nudE,Ptp69D,shn,stai,Wnk,Galphao,PlexA,Mical,nej,sif,dom,Pka-R2,zfh1,Gbeta13F,pod1,bun,ed,zip,Ten-a,dlg1,Myc,Dl,cno,Cam,chif,shot,poe,fax,Hrb27C,Appl,shg,clu,dally,rg,gish,Pka-C1,rin,trx,retn,nrm,RhoGAP100F,pum,ftz-f1,hdc,pAbp,Bsg,brat,sdk,dnc,Fmr1,unk,ct
GO:0009790	embryo development	49 out of 240 genes, 20.4%	544 out of 13900, 3.9%	7.70E-19	0.00%	kis,hth,dlp,p120ctn,AGO1,Top1,shn,osk,psq,Inx2,Syx1A,chrb,zfh1,Gbeta13F,tud,zip,ed,dlg1,Myc,Dl,cno,rdx,CG41099,shot,Plp,Cp190,Hrb27C,shg,dally,sog,sd,scyl,orb,Pka-C1,retn,kirre,Syt1,pum,ftz-f1,Ssdp,sqd,CtBP,gw,Fmr1,pyd,Rap1,cta,ct,lolal
GO:0009889	regulation of biosynthetic process	74 out of 240 genes, 30.8%	1307 out of 13900, 9.4%	7.12E-18	0.00%	Parp,skd,Not1,dpy,Sxl,eIF4EHP,psq,Atf6,Set2,Su(Tpl),Pdp1,Myc,srl,chif,Pcl,scrt,Sin3A,dally,Pka-C1,Syp,Usp7,crp,wisp,pum,ftz-f1,mamo,pAbp,brat,Su(z)2,CtBP,Trf2,msi,gw,Tis11,lolal,tim,hth,lid,kis,AGO1,shn,osk,MED26,PlexA,nej,upSET,zfh1,bun,dlg1,eIF4G1,Ncoa6,Cp190,Hrb27C,gpp,akirin,CG16779,sd,sog,orb,trx,Mnt,retn,E(bx),CG32767,Rbfox1,Nup153,Ssdp,sqd,Hrb98DE,bru1,Fmr1,CG4612,tou,ct
GO:0007389	pattern specification process	46 out of 240 genes, 19.2%	501 out of 13900, 3.6%	7.83E-18	0.00%	kis,Parp,hth,dlp,skd,AGO1,shn,osk,psq,Syx1A,nmo,dom,CG14073,Su(Tpl),bun,tucl,zip,ed,dlg1,Myc,Dl,cno,rdx,Hrb27C,shg,dally,sog,spoon,gish,orb,Pka-C1,Syp,rin,retn,Rbfox1,Syt1,pum,ftz-f1,pAbp,Ssdp,brat,sqd,CtBP,Fmr1,ct,lolal
GO:0051128	regulation of cellular component organization	56 out of 240 genes, 23.3%	777 out of 13900, 5.6%	2.56E-17	0.00%	kis,CG43658,CG11486,puf,RhoGAPp190,PlexB,mask,p120ctn,nudE,norpA,psq,Arpc2,stai,Galphao,PlexA,Syx1A,nej,upSET,sif,nmo,Gbeta13F,bun,Tao,ed,zip,Ten-a,dlg1,Myc,cno,srl,Pcl,shot,mgl,Cp190,Ncoa6,CaMKII,Slik,spoon,gish,DCP2,Pka-C1,kug,Syp,Mnt,kirre,Syt1,pum,mamo,brat,Su(z)2,bru1,Fmr1,pyd,cta,Rap1,tim

List contains the 16 most statistically significant overrepresented GO terms associated with genes that were downregulated by jetlag. GO terms have been filtered for redundancy and specificity (see methods and results). Cluster frequency refers to the number of genes corresponding to the GO term out of the total genes differentially downregulated (listed in rightmost column). Genome frequency refers to the relative number of genes in the fly genome associated with the corresponding GO term. False Discovery Rate (FDR) represents the likelihood of falsely identifying the GO term as overrepresented. Terms are listed from lowest p-value to highest. Comparison between cluster and genome frequency illustrates overrepresentation.

Table 2. Overrepresented GO terms among downregulated genes (nervous system).

Downregulated GO Terms (Nervous System)						
GO_ID	TERM	CLUSTER FREQUENCY	GENOME FREQUENCY	CORRECTED_PVALUE	FDR_RATE	ANNOTATED_GENES
GO:0007399	nervous system development	72 out of 240 genes, 30.0%	1082 out of 13900, 7.8%	2.15E-21	0.00%	dlp,mask,p120ctn,nudE,Ptp69D,stai,Galphao,Gbeta13F,pod1,zip,Ten-a,Myc,Dl,cno,chif,Pcl,poe,fax,Appl,shg,Sin3A,dally,Pka-C1,Syp,rin,nrm,RhoGAP100F,pum,ftz-f1,pAbp,brat,sdk,dnc,hth,kis,RhoGAPp190,PlexB,Ephrin,Rim,Reph,shep,AGO1,shn,Wnk,PlexA,nej,Mical,nmo,sif,dom,Pka-R2,zfh1,Tao,bun,ed,dlg1,Cam,shot,Hrb27C,CaMKII,clu,gish,rg,trx,retn,Rbfox1,hdc,Bsg,Fmr1,tou,unk,ct
GO:0022008	neurogenesis	62 out of 240 genes, 25.8%	862 out of 13900, 6.2%	1.51E-19	0.00%	kis,hth,dlp,RhoGAPp190,Ephrin,PlexB,mask,p120ctn,Rim,shep,nudE,Ptp69D,shn,stai,Wnk,Galphao,PlexA,Mical,nej,sif,dom,Pka-R2,zfh1,Gbeta13F,pod1,bun,ed,zip,Ten-a,dlg1,Myc,Dl,cno,Cam,chif,shot,poe,fax,Hrb27C,Appl,shg,clu,dally,rg,gish,Pka-C1,rin,trx,retn,nrm,RhoGAP100F,pum,ftz-f1,hdc,pAbp,Bsg,brat,sdk,dnc,Fmr1,unk,ct
GO:0061564	axon development	30 out of 240 genes, 12.5%	326 out of 13900, 2.3%	9.25E-11	0.00%	kis,dlp,Cam,chif,shot,fax,Hrb27C,RhoGAPp190,Ephrin,shg,PlexB,dally,nudE,Ptp69D,Pka-C1,trx,retn,Wnk,RhoGAP100F,PlexA,Mical,hdc,sif,brat,Pka-R2,zfh1,dnc,pod1,Fmr1,Ten-a
GO:0050808	synapse organization	23 out of 240 genes, 9.6%	283 out of 13900, 2.0%	1.13E-06	0.00%	kis,dlp,p120ctn,Appl,unc-13,CaMKII,AGO1,rg,Syp,dpr9,stai,RhoGAP100F,Galphao,pum,Mical,nej,nmo,brat,pod1,Fmr1,Ten-a,brp,dlg1
GO:0050890	cognition	17 out of 240 genes, 7.1%	159 out of 13900, 1.1%	3.34E-06	0.00%	kis,Appl,CaMKII,AGO1,spoon,rg,gish,Pka-C1,dikar,osk,Tob,pum,Rbfox1,dnc,Fmr1,CG4612,brp
GO:0007420	brain development	13 out of 240 genes, 5.4%	114 out of 13900, 0.8%	0.0001272	0.00%	hth,shot,ftz-f1,brat,RhoGAPp190,Ephrin,Appl,shg,Tao,bun,Fmr1,rg,Ten-a
GO:0007416	synapse assembly	14 out of 240 genes, 5.8%	165 out of 13900, 1.2%	0.001607909	0.00%	kis,stai,Galphao,pum,Mical,nej,nmo,brat,CaMKII,Fmr1,AGO1,Ten-a,dlg1,Syp
GO:0045475	locomotor rhythm	9 out of 240 genes, 3.8%	69 out of 13900, 0.5%	0.004237786	0.01%	Pka-R2,lid,gw,dnc,Fmr1,nej,Pka-C1,dlg1,tim
GO:0007417	central nervous system development	17 out of 240 genes, 7.1%	263 out of 13900, 1.9%	0.005049161	0.01%	hth,Pcl,shot,RhoGAPp190,Ephrin,Appl,shg,rg,Galphao,ftz-f1,brat,bun,Tao,Fmr1,Ten-a,Dl,ct
GO:0007268	chemical synaptic transmission	17 out of 240 genes, 7.1%	283 out of 13900, 2.0%	0.013422348	0.01%	cpo,Rim,unc-13,CaMKII,Pka-C1,Syp,pum,Syx1A,Syt1,nej,pAbp,nAChRalpha4,dnc,Fmr1,Ten-a,brp,dlg1

List contains 10 GO terms associated with downregulated genes associated with nervous system function. This list was only filtered by Revigo to remove redundant terms (see results). The FDR rate at 0% for all genes illustrates the high level of significance despite being excluded from table 1.

Table 3. Overrepresented GO terms among upregulated genes.

Upregulated Go Terms						
GO_ID	TERM	CLUSTER FREQUENCY	GENOME FREQUENCY	CORRECTED_PVALUE	FDR_RATE	ANNOTATED_GENES
GO:0009636	response to toxic substance	10 out of 104 genes, 9.6%	148 out of 13900, 1.1%	5.59E-05	0.00%	kraken,phu,CG8745,Drat,MtnE,Cyp6g2,Cat,Cyp12d1-p,Hsp70Bc,Cyp6a2
GO:0051186	cofactor metabolic process	12 out of 104 genes, 11.5%	247 out of 13900, 1.8%	0.000112806	0.00%	CG10365,GstO3,FeCH,GstE3,CG10096,Ugdo,ADX1,GstE1,Qtzl,Cat,Gnmt,FarO
GO:0051085	chaperone cofactor-dependent protein refolding	5 out of 104 genes, 4.8%	24 out of 13900, 0.2%	0.000294892	0.00%	Hsp70Bb,Hsp70Aa,Hsp70Bc,Hsc70-5,Hsp68
GO:0061077	chaperone-mediated protein folding	6 out of 104 genes, 5.8 %	44 out of 13900, 0.3%	0.000310378	0.00%	Hsp70Bb,Hsp70Aa,Hsp70Bc,Hsc70-5,Hsp22,Hsp68
GO:0006790	sulfur compound metabolic process	9 out of 104 genes, 8.7%	159 out of 13900, 1.14%	0.001060429	0.00%	CG10365,GstO3,GstE3,CG10096,GstE1,Eip71CD,Qtzl,Gnmt,FarO
GO:0009408	response to heat	7 out of 104 genes, 6.7%	88 out of 13900, 0.6%	0.001509776	0.00%	Hsp70Bb,GstE1,Hsp70Aa,Hsp70Bc,Hsc70-5,Hsp22,Hsp68
GO:0006986	response to unfolded protein	5 out of 104 genes, 4.8%	36 out of 13900, 0.3%	0.002435656	0.00%	Hsp70Bb,Hsp70Aa,Hsp70Bc,Hsc70-5,Hsp68
GO:0009266	response to temperature stimulus	8 out of 104 genes, 7.7%	144 out of 13900, 1.0%	0.004440108	0.17%	per,Hsp68,GstE1,Hsp70Bb,Hsp70Aa,Hsp70Bc,Hsp22,Hsc70-5
GO:0035966	response to topologically incorrect protein	5 out of 104 genes, 4.8%	46 out of 13900, 0.3%	0.008346132	0.27%	Hsp70Bb,Hsp70Aa,Hsp70Bc,Hsc70-5,Hsp68
GO:0008340	determination of adult lifespan	8 out of 104 genes, 7.7%	161 out of 13900, 1.2%	0.009976106	0.25%	per,Hsp68,Thor,Eip71CD,Cat,Hsp22,Gnmt,Tsp
GO:0035080	heat shock-mediated polytene chromosome puffing	3 out of 104 genes, 2.9%	9 out of 13900, 0.1%	0.012074269	0.22%	Hsp70Bb,Hsp70Aa,Hsp70Bc
GO:0046680	response to DDT	3 out of 104 genes, 2.9%	9 out of 13900, 0.1%	0.012074269	0.21%	FBgn0050489, FBgn0000473, FBgn0033696
GO:0035079	polytene chromosome puffing	3 out of 104 genes, 2.9%	10 out of 13900, 0.1%	0.017155109	0.38%	Hsp70Bb,Hsp70Aa,Hsp70Bc
GO:0006979	response to oxidative stress	7 out of 104 genes, 6.7%	132 out of 13900, 0.9%	0.021427666	0.64%	GstE1,Cat,Eip71CD,per,Hsp22,Thor,Sirup
GO:0001666	response to hypoxia	5 out of 104 genes, 4.8%	62 out of 13900, 0.4%	0.035838429	0.67%	Hsp70Bb,phu,Hsp70Aa,Drat,Hsp70Bc

List contains the 16 most statistically significant overrepresented GO terms associated with genes that were upregulated by jetlag. GO terms have been filtered for redundancy and specificity (see methods and results). Cluster frequency refers to the number of genes corresponding to the GO term out of the total genes differentially downregulated (listed in rightmost column). Genome frequency refers to the relative number of genes in the fly genome associated with the corresponding GO term. False Discovery Rate (FDR) represents the likelihood of falsely identifying the GO term as overrepresented. Terms are listed from lowest p-value to highest. Comparison between cluster and genome frequency illustrates overrepresentation.

APPENDIX A

LIST OF GENES DISPLAYING DIFFERENTIAL EXPRESSION, WEEKS 2 AND 3

Week 2														
Gene		Differential Expression						Normalized Expression						
ID	name	up/down	log2(FC)	std err	wald stat	p-value	p-adj	base mean	J_2W_A	J_2W_B	J_2W_C	C_2W_A	C_2W_B	C_2W_C
FBgn0038702	CG3739	DOWN	-1.26	0.19	6.78	1.17E-11	1.68E-07	3125.9	1993.5	1049.7	1382.7	4795.4	4666.7	4867.6
FBgn0037996	CG4830	DOWN	-1.14	0.21	5.51	3.68E-08	0.00026245	110.1	72.6	30.2	38.7	152.4	190.4	176.4
FBgn0024897	b6	DOWN	-1.04	0.19	5.35	9.01E-08	0.000428557	117.9	65.1	46.1	73.5	168.5	211.3	142.6
FBgn0034382	CG18609	DOWN	-0.80	0.16	4.91	8.97E-07	0.003201612	971.7	811.5	588.3	567.0	1330.9	1343.9	1188.5
FBgn0034629	Acox57D-d	DOWN	-0.66	0.14	4.87	1.13E-06	0.003236555	574.9	429.2	436.6	422.9	655.0	719.8	786.1
FBgn0038740	CG4562	DOWN	-0.65	0.15	4.40	1.10E-05	0.022367609	1904.7	1478.8	1399.3	1366.3	2198.0	2147.3	2838.6
FBgn0261925	CG42792	UP	0.88	0.19	-4.74	2.13E-06	0.005061661	12.7	11.7	22.2	41.6	0.9	0.0	0.0

Week 3														
Gene		Differential Expression Analysis						Normalized Expression						
ID	name	up/down	log2(FC)	std err	wald stat	p-value	p-adj	base mean	J_3W_A	J_3W_B	J_3W_C	C_3W_A	C_3W_B	C_3W_C
FBgn0038702	CG3739	DOWN	-0.90	0.11	8.42	3.68E-17	3.70E-13	3396.8	2230.7	2151.0	2458.4	4170.0	4933.2	4437.7
FBgn0034382	CG18609	DOWN	-0.80	0.10	8.25	1.54E-16	7.72E-13	1212.6	818.9	881.6	889.2	1554.6	1611.1	1519.9
FBgn0010052	Jhe	DOWN	-0.99	0.15	6.40	1.51E-10	5.06E-07	259.2	140.8	147.4	170.8	421.8	299.5	374.8
FBgn0036316	CG10960	DOWN	-0.67	0.11	6.19	5.93E-10	1.49E-06	9800.3	7191.3	6969.7	7933.3	13629.3	11464.6	11613.3
FBgn0015778	rin	DOWN	-0.65	0.11	5.93	3.07E-09	6.18E-06	1716.8	1241.3	1267.6	1396.3	2251.1	1920.5	2224.0
FBgn0052767	CG32767	DOWN	-0.78	0.14	5.60	2.16E-08	3.62E-05	200.2	136.2	128.1	147.9	263.4	266.9	258.6
FBgn0266557	kis	DOWN	-0.61	0.12	5.32	1.02E-07	0.000127851	456.2	325.1	359.7	365.5	570.0	563.4	553.7
FBgn0283657	Tlk	DOWN	-0.74	0.14	5.30	1.18E-07	0.000132163	329.4	200.1	237.7	253.0	392.0	414.1	479.7
FBgn0263396	sqd	DOWN	-0.52	0.11	4.93	8.04E-07	0.000577979	4690.7	3596.1	3673.9	4063.0	6116.6	5142.7	5552.0
FBgn0037248	srl	DOWN	-0.70	0.15	4.83	1.40E-06	0.000738617	229.3	168.6	155.3	163.5	284.0	262.9	341.8
FBgn0262124	uex	DOWN	-0.51	0.11	4.70	2.57E-06	0.00129163	683.2	575.3	546.5	538.3	865.3	780.8	793.0
FBgn0000273	Pka-C1	DOWN	-0.66	0.14	4.67	2.94E-06	0.001319355	517.0	347.4	339.5	445.7	742.8	593.0	633.5
FBgn0036814	CG14073	DOWN	-0.82	0.18	4.66	3.14E-06	0.001319355	84.6	38.9	58.8	54.1	117.3	110.7	127.6
FBgn0013733	shot	DOWN	-0.63	0.13	4.66	3.15E-06	0.001319355	943.0	663.3	673.7	782.0	1400.3	1072.4	1066.4
FBgn0023526	CG2865	DOWN	-0.63	0.14	4.63	3.68E-06	0.001447247	444.7	295.5	350.9	353.0	628.6	544.6	495.6
FBgn0085209	CG34180	DOWN	-0.75	0.16	4.61	3.97E-06	0.001457001	146.4	106.5	84.2	103.1	196.5	168.0	219.9
FBgn0038826	Syp	DOWN	-0.66	0.14	4.61	4.06E-06	0.001457001	862.4	663.3	536.0	685.1	1293.3	1078.4	918.3
FBgn0264493	rdx	DOWN	-0.64	0.14	4.59	4.50E-06	0.001558093	651.7	457.6	462.3	529.0	729.5	746.3	985.5
FBgn0000479	dnc	DOWN	-0.58	0.13	4.55	5.47E-06	0.00177453	615.3	461.3	471.1	495.6	872.5	700.8	690.4
FBgn0262656	Myc	DOWN	-0.74	0.16	4.50	6.66E-06	0.002080039	550.9	331.6	285.1	492.5	817.9	657.3	721.2
FBgn0003015	osk	DOWN	-0.58	0.13	4.50	6.82E-06	0.002080039	781.9	531.7	569.3	702.8	945.5	905.4	1036.8
FBgn0053196	dpy	DOWN	-0.77	0.17	4.47	7.95E-06	0.002221405	90.3	62.1	55.3	53.1	147.1	111.7	112.8
FBgn0260634	eIF4G2	DOWN	-0.65	0.15	4.45	8.41E-06	0.002287151	213.9	145.4	151.8	172.8	245.9	298.5	268.9
FBgn0039923	MED26	DOWN	-0.61	0.14	4.45	8.68E-06	0.002297637	357.1	265.9	252.6	287.4	516.5	411.2	409.0
FBgn0036165	chrb	DOWN	-0.60	0.14	4.41	1.04E-05	0.002612017	438.6	285.3	319.3	392.6	550.4	557.5	526.4
FBgn0001215	Hrb98DE	DOWN	-0.39	0.09	4.40	1.10E-05	0.002627896	3136.1	2645.7	2723.0	2692.7	3792.4	3406.1	3557.0
FBgn0031698	Ncoa6	DOWN	-0.67	0.15	4.31	1.61E-05	0.003681404	215.6	141.7	145.6	173.9	278.8	234.3	319.0
FBgn0035959	CG4911	DOWN	-0.65	0.15	4.28	1.90E-05	0.004156803	288.8	167.7	250.9	210.3	352.9	365.7	385.1
FBgn0259246	brp	DOWN	-0.77	0.18	4.24	2.23E-05	0.004590702	77.2	43.5	37.7	58.3	126.6	103.8	93.4
FBgn0261617	nej	DOWN	-0.61	0.14	4.24	2.28E-05	0.004590702	431.3	265.9	308.8	391.5	527.8	567.4	526.4
FBgn0004198	ct	DOWN	-0.72	0.17	4.23	2.31E-05	0.004590702	166.6	103.8	90.4	135.4	260.3	232.3	177.7
FBgn0034479	CG8654	DOWN	-0.54	0.13	4.22	2.47E-05	0.004787784	339.6	276.1	277.2	247.8	410.5	381.5	444.3
FBgn0034662	CG13492	DOWN	-0.62	0.15	4.18	2.88E-05	0.005271755	638.3	396.5	426.3	588.3	894.1	731.4	793.0
FBgn0039883	RhoGAP100F	DOWN	-0.74	0.18	4.18	2.93E-05	0.005271755	67.2	47.2	40.4	40.6	88.5	90.9	95.7
FBgn0004242	Syt1	DOWN	-0.65	0.16	4.16	3.20E-05	0.005638911	330.0	234.4	239.5	236.4	529.9	407.2	332.7
FBgn0003415	skd	DOWN	-0.62	0.15	4.11	3.91E-05	0.006336354	201.0	139.9	143.9	162.4	238.7	230.3	290.5
FBgn0000108	Appl	DOWN	-0.61	0.15	4.09	4.38E-05	0.006618558	374.1	263.1	251.8	320.7	549.4	460.6	398.8
FBgn0025741	PlexA	DOWN	-0.59	0.15	4.09	4.38E-05	0.006618558	459.8	340.9	318.4	379.0	695.5	524.8	500.2
FBgn0266521	stai	DOWN	-0.44	0.11	4.08	4.41E-05	0.006618558	712.9	599.3	553.5	630.0	826.2	862.9	805.5
FBgn0250869	CG42240	DOWN	-0.43	0.11	4.06	4.97E-05	0.007136459	764.6	629.9	679.0	609.1	938.3	874.7	856.8
FBgn0029932	CG4607	DOWN	-0.48	0.12	4.04	5.23E-05	0.007345192	3321.5	2329.8	2648.4	3120.6	3843.8	3899.3	4086.8
FBgn0026206	mei-P26	DOWN	-0.61	0.15	4.04	5.33E-05	0.007345192	259.4	200.1	166.7	211.4	343.6	359.8	274.6
FBgn0028734	Fmr1	DOWN	-0.55	0.14	4.04	5.33E-05	0.007345192	382.2	300.1	269.3	324.9	478.4	410.2	510.4
FBgn0010113	hdc	DOWN	-0.64	0.16	4.04	5.40E-05	0.007345192	236.4	127.8	178.1	204.1	299.4	308.4	300.8
FBgn0011481	Ssdp	DOWN	-0.55	0.14	4.02	5.83E-05	0.007704013	844.1	542.8	775.5	649.7	963.0	1032.9	1100.6
FBgn0264270	Sxl	DOWN	-0.51	0.13	4.02	5.92E-05	0.007704013	725.2	569.7	554.4	614.3	1009.3	817.4	786.1
FBgn0028863	CG4587	DOWN	-0.69	0.17	4.02	5.93E-05	0.007704013	100.5	63.9	65.8	72.9	158.4	131.5	110.5
FBgn0051221	CG31221	DOWN	-0.73	0.18	4.01	6.05E-05	0.007704013	354.0	200.1	171.9	277.0	719.2	383.5	372.6
FBgn0011666	msi	DOWN	-0.54	0.14	4.01	6.18E-05	0.007776791	353.4	255.7	322.0	248.9	424.9	443.8	425.0
FBgn0003862	trx	DOWN	-0.71	0.18	3.99	6.59E-05	0.008183756	151.7	90.8	69.3	135.4	215.0	196.7	202.8
FBgn0262739	AGO1	DOWN	-0.52	0.13	3.99	6.72E-05	0.008249932	1044.7	807.8	749.2	934.0	1443.5	1182.1	1151.9
FBgn0016694	Pdp1	DOWN	-0.63	0.16	3.98	6.89E-05	0.008354012	2023.6	1418.2	1305.3	1674.3	3412.7	2198.2	2132.8
FBgn0004838	Hrb27C	DOWN	-0.47	0.12	3.95	7.93E-05	0.009382238	6820.9	5097.7	5180.2	6436.0	8682.6	7397.3	8131.5
FBgn0025726	unc-13	DOWN	-0.51	0.13	3.91	9.06E-05	0.010597344	699.0	535.4	557.9	576.9	979.5	823.4	721.2
FBgn0000541	E(bx)	DOWN	-0.65	0.17	3.90	9.53E-05	0.011024172	213.0	117.6	136.9	190.5	266.5	246.1	320.2
FBgn0032946	nrv3	DOWN	-0.64	0.16	3.90	9.68E-05	0.011070958	1409.3	987.5	952.7	1072.5	2450.7	1684.3	1308.0
FBgn0011230	poe	DOWN	-0.55	0.14	3.89	1.00E-04	0.011095692	587.8	420.6	411.4	533.1	830.3	684.0	647.1
FBgn0037098	Wnk	DOWN	-0.66	0.17	3.89	0.000101578	0.011095692	129.5	88.0	67.5	111.4	182.1	164.1	164.1
FBgn0267033	mamo	DOWN	-0.60	0.15	3.88	0.000102762	0.011095692	426.0	306.6	276.3	363.4	663.6	473.5	472.8
FBgn0053100	4EHP	DOWN	-0.50	0.13	3.88	0.00010294	0.011095692	340.6	277.0	255.3	289.5	440.4	380.5	401.0
FBgn0023215	Mnt	DOWN	-0.69	0.18	3.88	0.000104163	0.011095692	153.3	74.1	93.0	134.3	171.8	210.5	235.8
FBgn0262730	dtn	DOWN	-0.53	0.14	3.88	0.000105446	0.011095692	208.6	170.4	155.3	164.5	268.5	248.1	245.0
FBgn0036111	Aps	DOWN	-0.50	0.13	3.88	0.000105883	0.011095692	847.2	653.1	664.1	717.4	1125.6	845.1	1077.8
FBgn0013343	Syx1A	DOWN	-0.67	0.17	3.85	0.000117277	0.011917768	111.7	69.5	71.1	86.4	180.1	120.6	142.4
FBgn0001105	Gbeta13F	DOWN	-0.51	0.13	3.85	0.000117282	0.011917768	2911.5	2160.2	2280.0	2534.4	4184.4	3251.9	3058.0
FBgn0038282	dpr9	DOWN	-0.69	0.18	3.85	0.000119637	0.011933257	99.0	59.3	62.3	69.8	175.9	104.8	121.9
FBgn0014037	Su(Tpl)	DOWN	-0.42	0.11	3.85	0.000119807	0.011933257	911.0	773.5	739.5	788.2	1134.8	964.7	1065.3
FBgn0033661	CG13185	DOWN	-0.71	0.18	3.83	0.000126986	0.012524327	65.2	31.5	33.3	53.1	87.5	80.1	106.0

FBgn0030869	Socs16D	DOWN	-0.39	0.10	3.83	0.000130624	0.012635382	851.8	706.8	761.5	711.2	1021.7	976.6	933.1
FBgn0032859	Arpc2	DOWN	-0.37	0.10	3.82	0.000134157	0.012853493	1048.0	884.7	908.0	925.7	1161.6	1168.3	1239.6
FBgn0041094	scyl	DOWN	-0.63	0.16	3.80	0.000144572	0.013374754	767.9	520.6	443.9	681.0	1257.3	797.7	906.9
FBgn0014396	tim	DOWN	-0.51	0.13	3.80	0.000144956	0.013374754	1690.2	1455.3	1258.9	1307.8	2384.9	1666.5	2067.9
FBgn0082598	akirin	DOWN	-0.45	0.12	3.80	0.000146245	0.013374754	1195.6	981.9	970.2	1005.8	1549.5	1201.9	1464.1
FBgn0035016	CG4612	DOWN	-0.44	0.12	3.79	0.000148487	0.013457476	572.0	431.7	487.8	506.0	699.6	640.5	666.5
FBgn0283499	InR	DOWN	-0.62	0.16	3.79	0.000150353	0.013504885	268.5	191.8	168.4	221.8	423.9	333.1	272.3
FBgn0031759	lid	DOWN	-0.45	0.12	3.78	0.000155853	0.013829177	765.3	583.6	632.5	685.1	964.0	817.4	909.2
FBgn0085436	Not1	DOWN	-0.54	0.14	3.78	0.000156712	0.013829177	918.5	658.6	677.2	811.1	1356.0	991.4	1016.3
FBgn0030504	CG2691	DOWN	-0.36	0.10	3.77	0.000165771	0.014501391	1078.5	914.3	949.2	942.3	1229.5	1173.3	1262.4
FBgn0263102	psq	DOWN	-0.42	0.11	3.75	0.000174823	0.015031758	615.8	481.7	504.4	563.3	705.8	736.4	703.0
FBgn0259212	cno	DOWN	-0.56	0.15	3.75	0.000176801	0.015072487	342.4	247.3	228.1	307.2	410.5	372.6	488.8
FBgn0011817	nmo	DOWN	-0.59	0.16	3.75	0.000180368	0.015072487	278.8	175.1	258.8	186.4	312.8	371.6	368.0
FBgn0052479	Usp10	DOWN	-0.50	0.13	3.74	0.000180413	0.015072487	433.3	297.4	357.0	381.1	527.8	473.5	562.8
FBgn0261822	Bsg	DOWN	-0.58	0.15	3.74	0.000181289	0.015072487	1664.0	1210.7	1096.6	1429.6	2670.9	1671.4	1905.0
FBgn0020306	dom	DOWN	-0.46	0.12	3.70	0.000213926	0.017405676	815.8	663.3	652.7	687.2	1042.2	813.5	1035.7
FBgn0052423	shep	DOWN	-0.48	0.13	3.70	0.000216027	0.017405676	911.7	691.1	710.6	814.3	1257.3	1018.1	978.7
FBgn0010247	Parp	DOWN	-0.52	0.14	3.69	0.000220483	0.017603613	268.7	206.6	221.1	206.2	373.5	315.3	289.4
FBgn0263706	CG43658	DOWN	-0.38	0.10	3.69	0.000225629	0.017872622	1077.7	931.9	891.3	947.5	1320.0	1148.5	1227.1
FBgn0022382	Pka-R2	DOWN	-0.49	0.13	3.69	0.000227534	0.017882735	527.9	424.3	371.1	476.9	702.7	605.9	586.8
FBgn0003891	tud	DOWN	-0.57	0.16	3.67	0.000244237	0.019046732	790.7	619.7	451.8	704.9	1176.0	876.7	914.9
FBgn0028397	Tob	DOWN	-0.64	0.17	3.66	0.000247433	0.019147502	157.0	115.8	113.2	95.8	259.3	208.6	149.3
FBgn0040324	Ephrin	DOWN	-0.47	0.13	3.66	0.000251723	0.019330815	533.8	463.2	443.9	396.7	715.1	602.9	581.1
FBgn0263987	spoon	DOWN	-0.62	0.17	3.66	0.000254359	0.019335176	336.4	225.1	188.6	304.0	508.3	339.0	453.5
FBgn0037636	CG9821	DOWN	-0.57	0.15	3.66	0.000255624	0.019335176	1957.5	1474.7	1148.3	1806.6	2923.0	2132.0	2260.4
FBgn0028704	Nckx30C	DOWN	-0.59	0.16	3.65	0.000259676	0.019495064	189.8	132.5	131.6	154.1	293.2	231.3	196.0
FBgn0029881	pigs	DOWN	-0.47	0.13	3.65	0.000263372	0.019626081	381.3	321.4	295.6	311.3	511.3	417.1	430.7
FBgn0000307	chif	DOWN	-0.62	0.17	3.64	0.000275209	0.020208787	113.8	83.4	72.8	84.3	151.2	117.6	173.2
FBgn0002778	mnd	DOWN	-0.43	0.12	3.63	0.000282376	0.020584783	913.2	682.7	736.9	859.0	1031.9	1033.9	1134.8
FBgn0000253	Cam	DOWN	-0.54	0.15	3.62	0.000295661	0.021094701	4329.0	3356.2	2810.7	3870.3	6467.4	4326.3	5143.0
FBgn0035397	CG11486	DOWN	-0.40	0.11	3.61	0.000306073	0.021532126	1273.7	958.8	1107.1	1166.2	1457.9	1402.6	1549.5
FBgn0011837	Tis11	DOWN	-0.58	0.16	3.58	0.000337556	0.023100742	657.9	492.8	400.0	563.3	1056.6	647.4	787.3
FBgn0004636	Rap1	DOWN	-0.45	0.13	3.57	0.000360749	0.024521188	1739.9	1364.5	1336.9	1600.4	2312.9	1793.0	2031.4
FBgn0086675	fne	DOWN	-0.66	0.19	3.56	0.000371917	0.025089551	113.8	70.4	67.5	73.9	234.6	123.6	112.8
FBgn0266098--FBgn0029746	rg	DOWN	-0.52	0.15	3.56	0.000374099	0.025089551	264.4	179.7	200.0	239.5	357.0	315.3	295.1
FBgn0003961	Uro	DOWN	-0.54	0.15	3.53	0.000414768	0.027451118	387.1	278.8	279.0	333.2	397.1	588.1	446.6
FBgn0036032	CG16711	DOWN	-0.41	0.12	3.53	0.000421469	0.027712258	404.5	347.4	326.3	349.9	458.9	462.6	481.9
FBgn0030366	Usp7	DOWN	-0.46	0.13	3.52	0.000427793	0.02773972	747.8	662.3	554.4	617.5	1020.6	825.3	806.7
FBgn0001235	hth	DOWN	-0.60	0.17	3.52	0.000428206	0.02773972	350.0	234.4	196.5	316.5	577.2	400.3	374.8
FBgn0085447	sif	DOWN	-0.51	0.15	3.51	0.00043976	0.028178253	266.7	210.3	186.0	232.2	352.9	341.0	278.0
FBgn0260799	p120ctn	DOWN	-0.45	0.13	3.51	0.000442812	0.028194246	759.2	634.5	582.5	654.9	1039.2	842.1	802.1
FBgn0004882	orb	DOWN	-0.51	0.15	3.51	0.000447643	0.02832258	459.5	315.9	352.7	416.5	470.2	627.6	574.2
FBgn0023213	eIF4G	DOWN	-0.56	0.16	3.51	0.000454455	0.028485235	853.8	636.4	510.6	783.0	1311.8	926.1	954.8
FBgn0086686	l(3)L1231	DOWN	-0.47	0.13	3.51	0.000455877	0.028485235	578.4	484.5	421.1	504.0	791.2	653.3	616.4
FBgn0262740	Evi5	DOWN	-0.53	0.15	3.50	0.000464526	0.028846466	185.0	139.9	130.7	160.4	242.8	201.6	234.7
FBgn0260970	Ubr3	DOWN	-0.40	0.11	3.50	0.000468133	0.028892129	475.0	406.7	379.8	422.7	561.8	545.6	533.2
FBgn0043884	mask	DOWN	-0.52	0.15	3.50	0.00047242	0.02890423	472.6	327.0	323.7	455.0	620.4	511.0	598.2
FBgn0053181	CG33181	DOWN	-0.42	0.12	3.49	0.000474075	0.02890423	852.3	636.4	817.6	681.0	1003.1	1005.2	970.7
FBgn0027492	wdb	DOWN	-0.49	0.14	3.49	0.000485356	0.029413735	517.1	401.1	358.8	480.0	702.7	567.4	592.5
FBgn0037137	Nopp140	DOWN	-0.46	0.13	3.48	0.000496143	0.02955541	1535.9	1223.7	1236.0	1290.1	2011.4	1451.0	2003.0
FBgn0011705	rost	DOWN	-0.51	0.15	3.48	0.000500202	0.02955541	1015.6	698.5	751.8	944.4	1464.1	1190.1	1044.8
FBgn0000463	Dl	DOWN	-0.62	0.18	3.48	0.000502383	0.02955541	79.9	50.9	57.0	55.2	81.3	110.7	124.2
FBgn0026375	RhoGAPp190	DOWN	-0.41	0.12	3.47	0.000511167	0.029732111	890.3	730.9	772.9	746.6	1166.7	988.4	936.5
FBgn0025709	CG8083	DOWN	-0.58	0.17	3.47	0.000517477	0.02991847	332.9	188.0	246.5	293.6	443.4	500.1	325.9
FBgn0250816	AGO3	DOWN	-0.64	0.18	3.46	0.000533171	0.030627386	206.7	76.0	174.6	151.0	223.3	303.4	312.2
FBgn0033010	Atf6	DOWN	-0.44	0.13	3.46	0.000538872	0.030627386	629.4	519.7	530.7	513.3	854.0	689.9	668.8
FBgn0037810	sle	DOWN	-0.63	0.18	3.41	0.000654035	0.035758637	70.3	39.8	45.6	51.0	67.9	98.8	118.5
FBgn0000547	ed	DOWN	-0.54	0.16	3.38	0.000713002	0.038563415	341.5	236.2	217.6	325.9	480.5	361.8	427.3
FBgn0033741	CG8545	DOWN	-0.52	0.15	3.37	0.000741257	0.039877232	514.2	420.6	354.4	420.7	738.7	477.4	673.3
FBgn0061200	Nup153	DOWN	-0.48	0.14	3.37	0.000759796	0.040229202	469.5	356.6	354.4	420.7	638.9	468.5	577.6
FBgn0261793	Trf2	DOWN	-0.52	0.16	3.37	0.000765327	0.040241305	174.1	140.8	119.3	142.7	249.0	194.7	198.2
FBgn0036735	Edc3	DOWN	-0.45	0.13	3.36	0.000788592	0.040892978	339.5	240.9	293.0	303.0	411.5	401.3	387.4
FBgn0020622	Pi3K21B	DOWN	-0.45	0.14	3.36	0.000793368	0.040929669	267.0	235.3	193.0	226.0	328.2	313.3	306.5
FBgn0261934	dikar	DOWN	-0.53	0.16	3.35	0.000814765	0.041819036	151.6	98.2	121.1	128.1	164.6	199.7	198.2
FBgn0037344	CG2926	DOWN	-0.48	0.14	3.34	0.000826022	0.041976653	354.3	257.5	263.2	332.2	411.5	387.5	474.0
FBgn0000114	bru1	DOWN	-0.39	0.12	3.34	0.000831081	0.041976653	2578.5	2058.3	2317.7	2209.5	2587.6	3287.5	3010.1
FBgn0014007	Ptp69D	DOWN	-0.58	0.17	3.34	0.000831098	0.041976653	81.9	63.9	58.8	54.1	113.2	91.9	109.4
FBgn0026533	Dek	DOWN	-0.43	0.13	3.34	0.000837139	0.041976653	1336.4	1119.0	1092.2	1113.1	1742.9	1275.1	1676.0
FBgn0262737	mub	DOWN	-0.39	0.12	3.34	0.000838699	0.041976653	1142.4	1020.8	959.7	930.9	1478.5	1288.9	1175.8
FBgn0264495	gpp	DOWN	-0.46	0.14	3.34	0.000851869	0.042265003	203.2	163.0	160.5	172.8	251.0	237.2	234.7

FBgn0024897	b6	DOWN	-0.55	0.17	3.34	0.000852862	0.042265003	150.1	117.6	88.6	130.2	209.9	186.8	167.5
FBgn0284408	trol	DOWN	-0.44	0.13	3.33	0.000883588	0.043353876	675.5	530.8	500.9	638.3	890.0	736.4	756.5
FBgn0029666	CG10803	DOWN	-0.52	0.16	3.32	0.000885154	0.043353876	152.5	107.5	122.8	123.9	169.8	178.9	211.9
FBgn0003391	shg	DOWN	-0.49	0.15	3.32	0.000890851	0.043353876	292.8	243.6	219.3	233.2	406.4	288.6	365.7
FBgn0285926	Imp	DOWN	-0.44	0.13	3.32	0.000896382	0.043353876	326.6	286.2	241.2	279.1	401.3	350.9	401.0
FBgn0029979	mahe	DOWN	-0.52	0.16	3.30	0.000953235	0.045664517	670.1	454.8	523.7	564.4	605.0	859.9	1012.9
FBgn0261260	mgl	DOWN	-0.36	0.11	3.30	0.000971884	0.045727423	779.6	645.7	669.3	703.9	942.4	899.5	816.9
FBgn0040531	CG11741	DOWN	-0.56	0.17	3.30	0.00097273	0.045727423	208.6	160.3	121.1	181.2	311.7	210.5	266.6
FBgn0030486	Set2	DOWN	-0.47	0.14	3.28	0.001024776	0.047727993	289.4	246.4	245.6	203.0	341.6	305.4	394.2
FBgn0004606	zfh1	DOWN	-0.48	0.15	3.27	0.001057832	0.048709303	262.7	217.7	194.7	215.5	347.8	260.9	339.5
FBgn0052062	Rbfox1	DOWN	-0.40	0.12	3.27	0.001078622	0.048890264	886.9	677.2	765.8	798.6	1159.5	967.7	952.5
FBgn0250823	gish	DOWN	-0.37	0.11	3.27	0.001078891	0.048890264	943.7	822.6	805.3	804.9	1150.3	1128.8	950.2
FBgn0039064	CG4467	DOWN	-0.54	0.16	3.27	0.001085704	0.048978415	143.1	110.2	107.9	105.2	219.1	167.0	149.3
FBgn0035424	CG11505	DOWN	-0.46	0.14	3.27	0.001092397	0.049060314	446.3	301.1	389.5	397.8	496.9	507.1	585.6
FBgn0036398	upSET	DOWN	-0.54	0.17	3.26	0.001103056	0.049318845	167.3	108.4	109.7	158.3	183.1	215.5	229.0
FBgn0020496	CtBP	DOWN	-0.30	0.09	3.24	0.001194474	0.052271698	3850.8	3207.0	3544.1	3522.5	4459.1	4282.8	4089.1
FBgn0022238	lolal	DOWN	-0.45	0.14	3.24	0.001200013	0.052271698	845.0	740.2	672.8	661.2	1209.9	867.8	918.3
FBgn0259736	CG42390	DOWN	-0.48	0.15	3.24	0.001202422	0.052271698	497.8	424.3	336.9	426.9	714.0	582.2	502.4
FBgn0259984	kuz	DOWN	-0.56	0.17	3.23	0.001216671	0.05230647	189.8	123.2	121.1	172.8	295.3	237.2	189.1
FBgn0020443	Elf	DOWN	-0.30	0.09	3.23	0.001240271	0.053013239	2802.8	2474.3	2454.5	2551.1	3158.6	2899.0	3279.0
FBgn0028369	kirre	DOWN	-0.51	0.16	3.20	0.001369794	0.057899703	115.0	83.4	99.1	83.3	138.9	140.4	144.7
FBgn0028743	Dhit	DOWN	-0.52	0.16	3.19	0.00142121	0.058971138	127.0	94.5	98.3	98.9	185.2	146.3	139.0
FBgn0001994	crp	DOWN	-0.37	0.11	3.19	0.001421232	0.058971138	1347.7	1094.0	1194.8	1186.0	1737.7	1453.0	1420.8
FBgn0004395	unk	DOWN	-0.38	0.12	3.19	0.001422077	0.058971138	469.8	374.2	410.6	415.5	584.4	525.8	508.1
FBgn0034087	clu	DOWN	-0.48	0.15	3.19	0.001429042	0.058971138	492.4	428.0	342.1	404.0	720.2	488.3	571.9
FBgn0010300	brat	DOWN	-0.55	0.17	3.19	0.001430314	0.058971138	393.3	214.9	381.6	271.8	405.4	490.3	595.9
FBgn0260780	wisp	DOWN	-0.60	0.19	3.18	0.001451241	0.05958973	176.7	76.9	148.3	105.2	141.0	240.2	348.6
FBgn0005666	bt	DOWN	-0.54	0.17	3.18	0.001459784	0.05969686	727.5	611.4	557.9	458.1	1002.1	1126.8	608.4
FBgn0001624	dlg1	DOWN	-0.37	0.12	3.18	0.001476432	0.060133222	671.9	547.5	626.4	552.9	825.1	766.0	713.2
FBgn0021764	sdk	DOWN	-0.50	0.16	3.17	0.001543	0.062591035	189.5	129.7	157.0	157.2	210.9	208.6	273.4
FBgn0034570	CG10543	DOWN	-0.52	0.17	3.16	0.001562009	0.062670644	239.1	175.1	142.1	228.0	333.4	255.0	300.8
FBgn0033638	CG9005	DOWN	-0.30	0.10	3.16	0.001563651	0.062670644	989.9	894.9	874.6	867.4	1094.7	1068.5	1139.3
FBgn0014163	fax	DOWN	-0.44	0.14	3.16	0.001573487	0.062774648	4263.6	3518.3	3292.3	3666.2	6313.1	4374.7	4417.2
FBgn0264607	CaMKII	DOWN	-0.43	0.14	3.16	0.001598911	0.063078595	1346.9	1103.3	1117.6	1113.1	1942.5	1462.9	1342.1
FBgn0265434	zip	DOWN	-0.52	0.16	3.15	0.001650324	0.064852559	512.2	372.4	300.0	497.7	761.4	536.7	605.0
FBgn0267001	Ten-a	DOWN	-0.49	0.16	3.14	0.00167122	0.065418165	176.2	134.3	114.9	165.6	217.1	207.6	217.6
FBgn0085412	CG34383	DOWN	-0.51	0.16	3.13	0.001722905	0.066920563	168.3	132.5	113.2	142.7	250.0	179.9	191.4
FBgn0027108	Inx2	DOWN	-0.28	0.09	3.13	0.001744771	0.06750922	1889.3	1653.5	1650.1	1779.5	2105.1	2108.3	2039.4
FBgn0266101	CG44838	DOWN	-0.45	0.15	3.12	0.001803151	0.068954428	228.6	184.3	207.0	163.5	246.9	276.8	292.8
FBgn0263354	CG42784	DOWN	-0.54	0.17	3.12	0.001803388	0.068954428	96.5	70.4	78.1	63.5	145.1	122.6	99.1
FBgn0029672	CG2875	DOWN	-0.52	0.17	3.12	0.001835672	0.069585463	170.8	132.5	127.2	126.0	256.2	156.2	226.7
FBgn0053208	Mical	DOWN	-0.41	0.13	3.11	0.001839934	0.069585463	441.7	349.2	407.0	347.8	522.7	570.3	453.5
FBgn0265623	Su(z)2	DOWN	-0.54	0.17	3.11	0.001873435	0.070323707	85.1	52.8	68.4	65.6	121.4	93.9	108.2
FBgn0003165	pum	DOWN	-0.35	0.11	3.11	0.001901753	0.070551851	1089.5	858.7	1040.4	931.9	1213.0	1185.1	1308.0
FBgn0261788--FBgn0265084	CG44195	DOWN	-0.58	0.19	3.10	0.001917649	0.070551851	69.8	28.7	65.8	40.6	100.8	92.9	90.0
FBgn0039214	puf	DOWN	-0.44	0.14	3.10	0.001930877	0.070551851	252.9	204.7	190.4	227.0	324.1	266.9	304.2
FBgn0003345	sd	DOWN	-0.46	0.15	3.10	0.001936632	0.070551851	406.7	312.2	309.7	358.2	599.8	432.9	427.3
FBgn0015024	CkIalpha	DOWN	-0.47	0.15	3.10	0.00194263	0.070551851	1431.2	1150.5	942.2	1325.5	2117.4	1420.4	1631.5
FBgn0000283	Cp190	DOWN	-0.53	0.17	3.10	0.001959454	0.070721824	261.2	168.6	175.4	241.6	383.8	241.2	356.6
FBgn0031632	CG15628	DOWN	-0.48	0.15	3.10	0.001961371	0.070721824	376.7	325.1	272.8	295.7	574.1	392.4	399.9
FBgn0026575	hang	DOWN	-0.48	0.16	3.09	0.001970875	0.070810711	267.1	177.9	208.8	243.7	281.9	305.4	385.1
FBgn0004880	scrt	DOWN	-0.56	0.18	3.09	0.002030377	0.072431182	73.3	50.9	39.5	62.5	113.2	93.9	79.8
FBgn0265297	pAbp	DOWN	-0.46	0.15	3.08	0.002044874	0.072505726	14058.0	11408.9	9565.5	12825.1	21261.4	13801.3	15485.9
FBgn0260943	Rbp6	DOWN	-0.52	0.17	3.08	0.002059786	0.072505726	154.5	116.7	125.4	107.2	245.9	163.1	168.6
FBgn0003277	RpII215	DOWN	-0.30	0.10	3.08	0.002068503	0.072505726	1146.9	969.9	1079.0	1005.8	1304.6	1270.1	1252.1
FBgn0036059	nudE	DOWN	-0.45	0.15	3.08	0.002097687	0.073273386	333.2	297.4	250.0	263.4	467.1	341.0	380.5
FBgn0031030	Tao	DOWN	-0.37	0.12	3.07	0.002108532	0.07339736	711.8	566.0	594.8	664.3	784.0	758.1	903.5
FBgn0003463	sog	DOWN	-0.37	0.12	3.07	0.002154636	0.074550367	877.6	694.8	754.4	800.7	1137.9	952.8	925.1
FBgn0041604	dlp	DOWN	-0.45	0.15	3.07	0.002156477	0.074550367	177.8	143.6	146.5	141.6	237.7	195.7	201.7
FBgn0266347	nAChRalpha4	DOWN	-0.50	0.16	3.07	0.002165631	0.074610423	117.1	88.9	102.6	80.2	150.2	148.3	132.2
FBgn0266410	CG45050	DOWN	-0.49	0.16	3.06	0.002183836	0.074955255	1639.0	1312.6	1027.3	1496.3	2580.4	1698.1	1719.3
FBgn0259176	bun	DOWN	-0.37	0.12	3.06	0.002204868	0.074955255	2770.8	2279.7	2180.8	2643.7	3601.0	2899.0	3020.4
FBgn0000384	cta	DOWN	-0.35	0.11	3.06	0.002205443	0.074955255	539.4	468.7	486.0	448.8	607.0	650.4	575.4
FBgn0033984	Lap1	DOWN	-0.51	0.17	3.06	0.002223595	0.075064989	99.7	75.0	67.5	85.4	116.3	118.6	135.6
FBgn0051992	gw	DOWN	-0.35	0.11	3.06	0.002241577	0.075418944	1737.4	1531.3	1479.0	1501.5	2255.3	1792.0	1865.1
FBgn0053547	Rim	DOWN	-0.53	0.17	3.05	0.002256467	0.075544799	98.7	70.4	69.3	80.2	150.2	119.6	102.5
FBgn0021800	Reph	DOWN	-0.42	0.14	3.05	0.002260336	0.075544799	335.1	260.3	251.8	319.7	429.0	370.7	379.4
FBgn0039955	CG41099	DOWN	-0.44	0.15	3.05	0.002272012	0.075683584	598.8	525.2	436.9	498.8	868.4	605.9	657.4
FBgn0041775	tral	DOWN	-0.41	0.13	3.05	0.002310534	0.076460419	3454.1	2545.6	3193.2	2905.1	3342.8	4093.0	4645.1
FBgn0267912	CanA-14F	DOWN	-0.29	0.09	3.04	0.002335844	0.076698018	2363.8	1997.2	2162.4	2183.5	2717.2	2483.9	2638.7

FBgn0037764	CG9459	DOWN	-0.35	0.11	3.03	0.002465582	0.080395518	537.5	442.8	477.2	479.0	643.0	616.8	566.3
FBgn0086690	Plp	DOWN	-0.45	0.15	3.03	0.002469405	0.080395518	278.6	228.8	194.7	249.9	388.9	294.5	314.5
FBgn0001078	ftz-f1	DOWN	-0.48	0.16	3.02	0.002515842	0.081380611	389.8	250.1	267.6	400.9	511.3	456.6	452.3
FBgn0261262	CG42613	DOWN	-0.51	0.17	3.02	0.002530119	0.081580125	241.0	179.7	143.0	223.9	368.3	244.1	287.1
FBgn0262509	nrm	DOWN	-0.54	0.18	3.02	0.002555466	0.081872558	80.0	68.5	52.6	50.0	126.6	88.0	94.6
FBgn0051104	CG31104	DOWN	-0.42	0.14	3.01	0.002583619	0.082227163	526.6	366.8	436.0	502.9	661.6	548.6	643.7
FBgn0003016	osp	DOWN	-0.49	0.16	3.01	0.002591055	0.082227163	156.5	129.7	101.8	135.4	207.8	164.1	200.5
FBgn0036816	Indy	DOWN	-0.24	0.08	3.01	0.002616043	0.082759106	4539.0	3986.1	4233.6	4197.3	4948.8	4952.0	4916.2
FBgn0259110	mmd	DOWN	-0.51	0.17	2.99	0.002748089	0.086663862	97.7	68.5	74.6	79.1	141.0	119.6	103.7
FBgn0020633	Mcm7	DOWN	-0.55	0.18	2.99	0.002807023	0.087889453	211.1	88.9	200.9	151.0	222.2	271.8	331.5
FBgn0261574	kug	DOWN	-0.54	0.18	2.99	0.002813161	0.087889453	66.5	39.8	43.0	59.4	94.7	80.1	82.0
FBgn0035001	Slik	DOWN	-0.35	0.12	2.98	0.002887155	0.089140701	656.1	591.9	543.9	564.4	823.1	676.1	737.2
FBgn0052369	CG32369	DOWN	-0.38	0.13	2.98	0.002904344	0.089140701	733.0	632.7	579.9	650.8	981.5	804.6	748.5
FBgn0029996	UbcE2H	DOWN	-0.41	0.14	2.98	0.002905616	0.089140701	1562.5	1294.1	1171.1	1432.8	2203.8	1566.6	1706.7
FBgn0036801	MYPT-75D	DOWN	-0.35	0.12	2.98	0.002906377	0.089140701	466.9	397.4	411.4	404.0	523.7	494.2	570.8
FBgn0036534	DCP2	DOWN	-0.49	0.16	2.97	0.002950134	0.089934374	316.3	225.1	193.9	316.5	431.1	330.1	401.0
FBgn0022764	Sin3A	DOWN	-0.37	0.13	2.97	0.003018227	0.091732215	829.0	740.2	642.1	739.3	1009.3	821.4	1022.0
FBgn0033636	tou	DOWN	-0.45	0.15	2.96	0.003072034	0.093086343	188.4	138.0	143.9	171.8	207.8	210.5	258.6
FBgn0004924	Top1	DOWN	-0.42	0.14	2.96	0.00310047	0.093665863	600.7	496.5	476.3	509.2	760.3	551.5	810.1
FBgn0263930	dally	DOWN	-0.40	0.14	2.95	0.003142478	0.094650687	486.3	398.3	407.0	416.5	671.8	516.0	508.1
FBgn0004795	retn	DOWN	-0.47	0.16	2.95	0.003181542	0.095258128	467.1	314.0	471.1	317.6	488.7	552.5	658.5
FBgn0262562	CG43102	DOWN	-0.43	0.14	2.95	0.003181584	0.095258128	340.8	306.6	254.4	279.1	474.3	370.7	360.0
FBgn0001122	Galphao	DOWN	-0.48	0.16	2.95	0.003214249	0.095950571	353.3	239.0	277.2	314.5	538.1	336.1	414.7
FBgn0025740	PlexB	DOWN	-0.45	0.15	2.94	0.003254494	0.096785869	311.2	236.2	226.3	287.4	449.6	349.9	317.9
FBgn0262743	Fs(2)Ket	DOWN	-0.50	0.17	2.94	0.003265932	0.096785869	279.7	203.8	228.1	210.3	271.6	298.5	466.0
FBgn0262738	norpA	DOWN	-0.41	0.14	2.94	0.003280714	0.096785869	457.7	378.9	379.0	387.3	638.9	474.4	487.6
FBgn0033000	CG14464	DOWN	-0.33	0.11	2.94	0.00332227	0.097725245	664.6	608.6	550.9	586.2	793.3	751.2	697.3
FBgn0003396	shn	DOWN	-0.52	0.18	2.94	0.003332461	0.097739237	123.3	73.2	78.1	121.8	163.6	135.4	167.5
FBgn0037698	CG16779	DOWN	-0.53	0.18	2.93	0.003364052	0.098378968	74.9	48.2	52.6	61.4	111.1	101.8	74.1
FBgn0030148	CG3106	DOWN	-0.44	0.15	2.93	0.003408728	0.099027922	411.7	281.6	404.4	317.6	453.7	553.5	459.2
FBgn0005777	PpD3	DOWN	-0.29	0.10	2.93	0.003433612	0.099027922	726.0	638.3	665.8	636.2	812.8	805.6	797.5
FBgn0010100	Acon	DOWN	-0.30	0.10	2.93	0.003434723	0.099027922	24387.1	20791.9	22582.1	21522.6	29047.9	28225.2	24152.9
FBgn0034394	CG15096	DOWN	-0.46	0.16	2.93	0.003443236	0.099027922	11980.3	7965.7	7787.3	12759.5	14679.8	12575.6	16113.7
FBgn0003044	Pcl	DOWN	-0.42	0.14	2.92	0.003447366	0.099027922	291.3	236.2	222.8	259.3	310.7	314.3	404.5
FBgn0029903	pod1	DOWN	-0.36	0.12	2.92	0.003459647	0.099027922	527.3	409.4	495.6	450.9	553.5	636.5	617.5
FBgn0263995	cpo	DOWN	-0.49	0.17	2.92	0.003475629	0.099027922	399.8	299.2	305.3	324.9	625.5	503.1	340.7
FBgn0262614	pyd	DOWN	-0.28	0.09	2.92	0.003483607	0.099027922	1064.2	931.9	959.7	974.6	1213.0	1157.4	1148.5

Week 3														
Gene		Differential Expression Analysis						Normalized Expression						
ID	name	up/down	log2(FC)	std err	wald stat	p-value	p-adj	base mean	J_3W_A	J_3W_B	J_3W_C	C_3W_A	C_3W_B	C_3W_C
FBgn0036110	Cpr67Fb	UP	0.79	0.15	-5.37	7.94E-08	0.000114131	248.0	377.0	307.9	299.9	158.4	159.1	185.7
FBgn0063497	GstE3	UP	0.55	0.11	-5.19	2.11E-07	0.00020348	861.3	1012.5	1011.5	1092.3	653.3	664.2	733.7
FBgn0035670	CG10472	UP	0.43	0.08	-5.18	2.22E-07	0.00020348	13910.7	15799.8	16635.3	15777.0	11243.4	12168.4	11840.0
FBgn0051323--FBgn0051086	CG31086	UP	0.54	0.11	-5.16	2.43E-07	0.000203667	1043.3	1332.1	1276.4	1155.8	808.7	822.4	864.8
FBgn0034335	GstE1	UP	0.56	0.11	-4.94	7.99E-07	0.000577979	672.8	768.9	807.9	870.5	551.5	490.3	548.0
FBgn0035343	CG16762	UP	0.85	0.17	-4.91	8.97E-07	0.000598011	5732.1	5513.6	7089.0	11411.1	3360.3	3044.3	3974.0
FBgn0040993--FBgn0259201	CG17325	UP	0.73	0.15	-4.90	9.51E-07	0.000598011	2845.9	3522.9	3151.1	4468.0	2028.9	1597.3	2307.2
FBgn0036837	CG18135	UP	0.46	0.09	-4.86	1.17E-06	0.000670708	24237.7	28906.7	25865.7	30215.0	19559.7	20343.6	20535.5
FBgn0033188	Drat	UP	0.44	0.09	-4.86	1.20E-06	0.000670708	8594.8	9636.8	10448.0	9815.9	6895.4	7656.3	7116.3
FBgn0042086--FBgn0050160	Tsp42Eb	UP	0.63	0.13	-4.69	2.71E-06	0.001298929	1641.2	1769.3	2378.2	2016.9	1089.6	1387.7	1205.4
FBgn0037478	CG2656	UP	0.41	0.09	-4.63	3.74E-06	0.001447247	4583.9	5248.7	5005.6	5533.2	4025.9	3822.2	3868.1
FBgn0028533	CG7953	UP	0.48	0.11	-4.58	4.65E-06	0.001558093	1411.1	1639.6	1535.2	1829.5	1176.0	1167.3	1118.8
FBgn0040775	CG12158	UP	0.72	0.16	-4.49	7.04E-06	0.002082	1187.0	1610.9	1965.0	1114.1	867.3	850.0	714.4
FBgn0053192	MtnD	UP	0.69	0.15	-4.48	7.36E-06	0.002115758	227.4	283.5	265.8	334.2	171.8	132.4	176.6
FBgn0023550	CG18031	UP	0.46	0.10	-4.41	1.05E-05	0.002612017	718.2	790.2	867.6	870.5	586.5	601.9	592.5
FBgn0031726	Cyp6a16	UP	0.77	0.18	-4.40	1.06E-05	0.002612017	95.8	105.6	136.9	153.1	53.5	58.3	67.2
FBgn0031277	CG13947	UP	0.56	0.13	-4.34	1.40E-05	0.003270464	3437.6	4069.5	3869.5	4636.7	2446.6	2438.4	3165.1
FBgn0051354--FBgn0013279	Hsp70Bc	UP	0.80	0.19	-4.29	1.78E-05	0.003971679	122.4	155.6	258.8	116.6	66.9	67.2	69.5
FBgn0036381	CG8745	UP	0.55	0.13	-4.24	2.26E-05	0.004590702	11479.9	13210.7	11871.8	16949.5	8579.7	9479.9	8787.7
FBgn0031971	Sirup	UP	0.53	0.12	-4.23	2.33E-05	0.004590702	5983.5	7825.8	6426.7	7404.3	4888.1	4054.5	5301.3
FBgn0032087	CG9568	UP	0.58	0.14	-4.20	2.66E-05	0.005041333	955.7	1218.2	1232.5	1086.0	734.6	864.9	598.2
FBgn0035231	Cct2	UP	0.41	0.10	-4.18	2.86E-05	0.005271755	1041.3	1233.0	1141.3	1221.4	896.1	857.9	897.8
FBgn0250836	CG8628	UP	0.44	0.11	-4.15	3.31E-05	0.005747804	606.2	717.9	724.6	679.9	526.8	491.2	496.8
FBgn0043806	CG32032	UP	0.43	0.10	-4.13	3.55E-05	0.006061409	1426.0	1710.0	1508.9	1744.1	1244.9	1171.3	1176.9
FBgn0043791	phu	UP	0.62	0.15	-4.12	3.84E-05	0.006336354	6389.4	7230.2	6757.4	10278.2	4115.4	4463.7	5491.6
FBgn0040609	CG3348	UP	0.54	0.13	-4.12	3.87E-05	0.006336354	679.7	871.7	848.3	758.0	464.0	617.8	518.4
FBgn0051106	CG31106	UP	0.61	0.15	-4.10	4.05E-05	0.006474805	325.6	386.3	392.1	446.7	285.0	245.1	198.2
FBgn0038074	Gnmt	UP	0.60	0.15	-4.09	4.37E-05	0.006618558	9866.0	12459.4	10350.7	14198.5	6390.3	6676.8	9120.4
FBgn0000473	Cyp6a2	UP	0.41	0.10	-4.08	4.48E-05	0.006628306	681.2	795.7	769.3	792.4	578.2	569.3	582.2
FBgn0029838	CG4666	UP	0.39	0.10	-4.06	4.84E-05	0.007063645	1459.9	1637.8	1790.5	1588.9	1258.3	1237.5	1246.4
FBgn0035926	CG5804	UP	0.51	0.13	-4.01	6.01E-05	0.007704013	2106.2	2638.2	2570.3	2382.4	1509.3	1528.1	2008.7
FBgn0000565	Eip71CD	UP	0.57	0.14	-3.97	7.05E-05	0.008445497	2507.9	3864.7	2514.2	2905.1	1818.0	1904.7	2040.6
FBgn0036024	CG18180	UP	0.44	0.11	-3.86	0.000112021	0.011617819	3405.6	3524.8	3859.0	4538.8	2812.9	2854.5	2843.8
FBgn0033297	Mal-A8	UP	0.57	0.15	-3.83	0.000130028	0.012635382	1534.8	1762.8	1672.0	2264.7	1155.4	1389.7	963.9
FBgn0035189	CG9119	UP	0.56	0.15	-3.81	0.000141693	0.013374754	2673.4	2834.6	2834.4	4257.7	1902.4	1990.7	2220.6
FBgn0039452	CG14245	UP	0.69	0.18	-3.80	0.000143429	0.013374754	1340.5	1363.6	1244.8	2850.9	859.1	883.6	840.8
FBgn0029580	CG14778	UP	0.53	0.14	-3.76	0.000170092	0.014751039	191.7	236.2	238.6	223.9	154.3	142.3	155.0
FBgn0263621	CG43630	UP	0.62	0.17	-3.71	0.000205986	0.016985368	316.6	378.9	410.6	431.1	201.7	172.0	305.3
FBgn0036183	CG6083	UP	0.45	0.12	-3.70	0.000216273	0.017405676	362.8	446.5	435.1	397.8	309.7	298.5	289.4
FBgn0267408	AOX1	UP	0.38	0.10	-3.65	0.000266482	0.019711836	7539.7	7730.4	8841.8	9296.3	6392.3	6839.8	6137.6
FBgn0050360	Mal-A6	UP	0.50	0.14	-3.63	0.000287787	0.02082836	8633.8	8459.4	10270.8	12414.8	6555.9	7661.2	6440.7
FBgn0019928	Ser8	UP	0.59	0.16	-3.62	0.000290185	0.020851894	339.8	378.9	372.8	539.4	294.3	207.6	246.1
FBgn0051547	CG31547	UP	0.35	0.10	-3.62	0.000299239	0.021199573	1273.0	1404.3	1451.8	1451.5	1048.4	1157.4	1124.5
FBgn0025814	Mgstl	UP	0.32	0.09	-3.60	0.000315054	0.022010031	3463.5	3793.4	3819.5	3997.4	2881.8	3134.3	3154.8
FBgn0030895	CG7135	UP	0.52	0.14	-3.59	0.000333416	0.023032262	234.7	257.5	275.5	323.8	197.5	169.0	184.6
FBgn0261508	CG42656	UP	0.53	0.15	-3.59	0.000334265	0.023032262	1468.4	1840.7	1905.4	1650.4	928.0	1030.9	1454.9
FBgn0037166	CG11426	UP	0.36	0.10	-3.56	0.000377562	0.025154103	1317.0	1406.2	1457.1	1622.3	1154.4	1114.9	1147.3
FBgn0053532	lectin-37Da	UP	0.64	0.18	-3.52	0.000430159	0.02773972	1008.4	1372.9	1336.1	1346.3	820.0	838.2	337.2
FBgn0031068	Alr	UP	0.41	0.12	-3.48	0.000498466	0.02955541	1103.3	1456.2	1193.9	1178.7	924.9	910.3	955.9
FBgn0051354--FBgn0013278	Hsp70Bb	UP	0.64	0.19	-3.48	0.000498838	0.02955541	140.6	191.8	271.9	113.5	84.4	90.9	91.1
FBgn0033204	CG2065	UP	0.50	0.14	-3.47	0.000511298	0.029732111	239.5	285.3	301.8	281.1	172.8	221.4	174.3
FBgn0028978	trbl	UP	0.36	0.10	-3.45	0.000550402	0.031107	1163.1	1221.9	1389.6	1345.3	997.0	969.6	1055.0
FBgn0085359	CG34330	UP	0.41	0.12	-3.45	0.000557974	0.031358787	2153.0	2142.6	2692.3	2651.0	1706.9	1943.2	1781.9
FBgn0035904	GstO3	UP	0.30	0.09	-3.44	0.000575536	0.032132183	2045.6	2255.7	2247.5	2312.6	1890.0	1751.5	1816.1
FBgn0032162	CG4592	UP	0.40	0.12	-3.44	0.000578124	0.032132183	720.1	783.7	842.2	864.2	597.8	560.4	672.2
FBgn0027521--FBgn0030737	CG9914	UP	0.53	0.16	-3.42	0.000634086	0.035048944	978.5	1144.0	930.8	1540.0	700.7	720.6	835.1
FBgn0028920	CG8997	UP	0.39	0.12	-3.41	0.000652495	0.035758637	2073.5	2447.4	2059.8	2656.2	1797.4	1713.9	1766.0
FBgn0000261	Cat	UP	0.29	0.09	-3.39	0.00070915	0.038562401	27729.3	30778.9	30070.3	31184.4	26343.0	23473.9	24525.4
FBgn0039486	caix	UP	0.32	0.10	-3.37	0.000748941	0.040076337	1116.7	1250.6	1258.0	1244.3	930.1	1025.0	992.4
FBgn0040060	yip7	UP	0.37	0.11	-3.37	0.000754431	0.040156486	12039.2	13255.1	15713.3	12300.3	10567.4	10035.4	10363.4
FBgn0032088	CG13102	UP	0.53	0.16	-3.36	0.000768025	0.040241305	169.2	216.8	206.2	204.1	156.4	107.7	124.2
FBgn0037071	CG7632	UP	0.34	0.10	-3.36	0.000772577	0.040270064	973.8	1162.6	1050.1	1077.7	871.4	826.3	854.5
FBgn0261560	Thor	UP	0.34	0.10	-3.32	0.000892747	0.043353876	10741.5	11739.6	11431.4	13117.7	10073.6	8760.3	9326.6
FBgn0053296	CG33296	UP	0.43	0.13	-3.32	0.000914419	0.044014624	261.8	298.3	302.7	320.7	214.0	203.6	231.3
FBgn0031523	CG15408	UP	0.54	0.16	-3.30	0.000969183	0.045727423	234.6	253.8	269.3	353.0	150.2	167.0	214.2
FBgn0039109	CG10365	UP	0.26	0.08	-3.30	0.000971871	0.045727423	4845.8	5285.8	5225.8	5371.8	4335.6	4412.3	4443.4
FBgn0003068	per	UP	0.35	0.10	-3.29	0.000989924	0.04631924	1095.1	1194.1	1288.7	1229.7	875.6	968.6	1014.0
FBgn0053533	lectin-37Db	UP	0.35	0.11	-3.28	0.001053096	0.048709303	886.0	1023.6	921.1	1065.2	756.2	757.1	793.0
FBgn0034292	CG5767	UP	0.45	0.14	-3.27	0.001060372	0.048709303	1279.2	1422.9	1742.2	1366.1	1189.4	1062.5	892.1
FBgn0039184	CG6432	UP	0.38	0.12	-3.27	0.001069528	0.048890264	1183.1	1172.8	1438.7	1466.1	1047.4	981.5	992.4
FBgn0004431	LysX	UP	0.60	0.18	-3.25	0.001143809	0.050808323	138.6	167.7	224.6	161.4	134.8	75.1	68.4

FBgn0038658	CG14292	UP	0.46	0.14	-3.25	0.00114647	0.050808323	20348.2	20182.4	21801.4	30564.8	15876.4	16346.4	17318.0
FBgn0031490	CG17264	UP	0.49	0.15	-3.25	0.001159162	0.051145473	264.9	309.4	332.5	319.7	238.7	225.4	164.1
FBgn0032669	CG15155	UP	0.52	0.16	-3.24	0.001210667	0.052271698	144.9	171.4	209.7	155.1	102.9	110.7	119.6
FBgn0001228--FBgn0001223	Hsp22	UP	0.55	0.17	-3.23	0.001243651	0.053013239	41505.3	71986.6	36941.8	47796.5	28185.7	27149.8	36971.6
FBgn0030737	CG9914	UP	0.51	0.16	-3.21	0.001338108	0.056799015	3893.0	4190.8	3816.0	6338.1	2653.4	2850.6	3509.2
FBgn0038032--FBgn0038033	CG10096	UP	0.41	0.13	-3.19	0.001426986	0.058971138	8913.9	11381.1	11105.1	8568.4	7854.3	7873.7	6700.5
FBgn0050489--FBgn0053503	Cyp12d1-p	UP	0.42	0.13	-3.16	0.001557979	0.062670644	624.6	800.4	625.5	762.2	575.1	485.3	499.0
FBgn0020545	kraken	UP	0.34	0.11	-3.16	0.001579168	0.062774648	4356.7	4645.6	4836.3	5259.4	3447.7	3790.6	4160.9
FBgn0036136	Ufd1-like	UP	0.32	0.10	-3.16	0.001584966	0.062774648	1507.0	1793.4	1574.7	1689.9	1366.3	1263.2	1354.7
FBgn0262146	MtnE	UP	0.50	0.16	-3.14	0.001690958	0.065934271	2480.3	2685.5	2589.6	3832.8	1882.8	1528.1	2363.0
FBgn0034909	CG4797	UP	0.37	0.12	-3.12	0.001790475	0.068954428	1049.7	1146.8	1130.8	1322.4	860.1	840.2	998.1
FBgn0001220	Hsc70-5	UP	0.32	0.10	-3.12	0.00180954	0.068954428	18523.6	22587.2	21008.4	18550.9	16417.5	16690.4	15886.9
FBgn0260747	CG5010	UP	0.27	0.09	-3.11	0.001867858	0.070323707	54581.8	61379.9	56299.2	62139.7	50003.7	47371.8	50296.1
FBgn0034605	CG15661	UP	0.41	0.13	-3.11	0.001886326	0.070544388	561.6	665.1	551.8	746.6	466.1	460.6	479.7
FBgn0051864--FBgn0032393	Qtzl	UP	0.36	0.12	-3.11	0.001893683	0.070551851	742.3	831.9	872.0	833.0	627.6	576.2	713.2
FBgn0027564	CG3149	UP	0.29	0.09	-3.10	0.001936064	0.070551851	1482.2	1572.9	1633.4	1710.8	1292.3	1338.3	1345.6
FBgn0260933--FBgn0031263	Tspo	UP	0.30	0.10	-3.10	0.001940649	0.070551851	3142.5	3586.8	3368.6	3538.2	2726.5	2604.5	3030.6
FBgn0266268	FeCH	UP	0.30	0.10	-3.09	0.002030335	0.072431182	2559.2	2918.9	2716.8	2910.3	2453.8	2135.0	2220.6
FBgn0038083	CG5999	UP	0.50	0.16	-3.08	0.002047075	0.072505726	126.3	141.7	169.3	153.1	99.8	108.7	85.5
FBgn0010387	Dbi	UP	0.42	0.14	-3.08	0.002068363	0.072505726	5667.4	6696.6	6026.7	7210.7	4370.6	3965.5	5734.3
FBgn0050269--FBgn0050273	CG30269	UP	0.30	0.10	-3.06	0.002196416	0.074955255	1301.4	1364.5	1468.5	1512.9	1193.5	1099.1	1170.1
FBgn0037378	CG2046	UP	0.31	0.10	-3.06	0.002215114	0.075030456	1132.8	1265.4	1251.8	1273.4	921.9	1042.8	1041.4
FBgn0041607	AsnS	UP	0.37	0.12	-3.05	0.002284281	0.075841159	1169.7	1220.0	1422.9	1376.5	931.1	927.1	1140.5
FBgn0001230	Hsp68	UP	0.57	0.19	-3.04	0.002335309	0.076698018	483.0	567.9	1056.2	339.4	267.5	383.5	283.7
FBgn0013275--FBgn0013276	Hsp70Aa	UP	0.47	0.15	-3.04	0.002340586	0.076698018	2038.7	2813.3	6491.6	1003.8	609.1	635.6	679.0
FBgn0033428	Updo	UP	0.28	0.09	-3.03	0.002479156	0.080452616	2333.7	2652.1	2476.5	2586.5	2043.3	2031.2	2212.6
FBgn0033928	Arc2	UP	0.30	0.10	-3.02	0.00254783	0.081872558	1400.9	1546.1	1472.0	1654.5	1192.5	1247.4	1293.2
FBgn0083972	CG34136	UP	0.43	0.14	-3.01	0.002587644	0.082227163	445.5	552.1	445.6	577.9	420.8	343.0	333.8
FBgn0016123	Alp4	UP	0.41	0.14	-2.99	0.002781981	0.087458536	4752.7	5384.9	5141.6	6109.0	3213.1	3956.6	4711.2
FBgn0039311	CG10513	UP	0.28	0.09	-2.98	0.002841148	0.088489004	1128.4	1228.3	1238.7	1263.0	1042.2	993.4	1004.9
FBgn0035176	CG13905	UP	0.48	0.16	-2.98	0.002873022	0.089140701	2066.9	2029.6	2222.9	3286.2	1678.1	1864.2	1320.5
FBgn0028583	Ics	UP	0.47	0.16	-2.97	0.002930364	0.089603216	54219.9	69820.8	56296.6	70219.8	37855.9	33991.6	57134.4
FBgn0033696	Cyp6g2	UP	0.46	0.16	-2.94	0.003271791	0.096785869	315.2	365.9	323.7	447.7	264.4	204.6	284.8
FBgn0036831	CG6839	UP	0.46	0.16	-2.92	0.00345883	0.099027922	10515.8	12564.1	10312.9	15208.5	8086.9	6431.6	10491.0
FBgn0259992	CG42489	UP	0.49	0.17	-2.92	0.00348468	0.099027922	101.7	128.8	113.2	132.2	84.4	68.2	83.2

APPENDIX B

LIST OF GO TERMS,

WEEK 3

Downregulated Go Terms (Full List)						
GO_ID	TERM	GO Hierarchy Level	CLUSTER FREQUENCY	GENOME FREQUENCY	CORRECTED_PVALUE	FDR_RATE
GO:0048856	anatomical structure development	3	139 out of 240 genes, 57.9%	2703 out of 13900, 19.4%	5.32E-37	0.00%
GO:0032502	developmental process	2	141 out of 240 genes, 58.8%	2826 out of 13900, 20.3%	3.04E-36	0.00%
GO:0048869	cellular developmental process	3	109 out of 240 genes, 45.4%	1734 out of 13900, 12.5%	5.90E-34	0.00%
GO:0050794	regulation of cellular process	3	152 out of 240 genes, 63.3%	3470 out of 13900, 24.9%	1.61E-33	0.00%
GO:0050789	regulation of biological process	3	158 out of 240 genes, 65.8%	3783 out of 13900, 27.2%	4.49E-33	0.00%
GO:0048468	cell development	4	97 out of 240 genes, 40.4%	1397 out of 13900, 10.1%	1.02E-32	0.00%
GO:0030154	cell differentiation	4	106 out of 240 genes, 44.2%	1688 out of 13900, 12.1%	1.13E-32	0.00%
GO:0007275	multicellular organism development	4	123 out of 240 genes, 51.3%	2346 out of 13900, 16.9%	1.02E-31	0.00%
GO:0065007	biological regulation	2	164 out of 240 genes, 68.3%	4221 out of 13900, 30.4%	4.50E-31	0.00%
GO:0009653	anatomical structure morphogenesis	4	98 out of 240 genes, 40.8%	1512 out of 13900, 10.9%	1.29E-30	0.00%
GO:0048731	system development	5	102 out of 240 genes, 42.5%	1722 out of 13900, 12.4%	7.36E-29	0.00%
GO:0032501	multicellular organismal process	2	147 out of 240 genes, 61.3%	3642 out of 13900, 26.2%	1.80E-27	0.00%
GO:0016043	cellular component organization	3	122 out of 240 genes, 50.8%	2866 out of 13900, 20.6%	1.82E-22	0.00%
GO:0071840	cellular component organization or biogenesis	2	124 out of 240 genes, 51.7%	2994 out of 13900, 21.5%	6.80E-22	0.00%
GO:0048513	animal organ development	6	76 out of 240 genes, 31.7%	1197 out of 13900, 8.6%	1.39E-21	0.00%
GO:0019222	regulation of metabolic process	3	100 out of 240 genes, 41.7%	2038 out of 13900, 14.7%	1.86E-21	0.00%
GO:0007399	nervous system development	6	72 out of 240 genes, 30.0%	1082 out of 13900, 7.8%	2.15E-21	0.00%
GO:0048518	positive regulation of biological process	2	83 out of 240 genes, 34.6%	1447 out of 13900, 10.4%	4.21E-21	0.00%
GO:0010468	regulation of gene expression	6	84 out of 240 genes, 35.0%	1492 out of 13900, 10.7%	7.02E-21	0.00%
GO:0009888	tissue development	4	69 out of 240 genes, 28.8%	1047 out of 13900, 7.5%	4.72E-20	0.00%
GO:0048522	positive regulation of cellular process	3	76 out of 240 genes, 31.7%	1277 out of 13900, 9.2%	7.94E-20	0.00%
GO:0032989	cellular component morphogenesis	5	55 out of 240 genes, 22.9%	668 out of 13900, 4.8%	1.11E-19	0.00%
GO:0022008	neurogenesis	7	62 out of 240 genes, 25.8%	862 out of 13900, 6.2%	1.51E-19	0.00%
GO:0048523	negative regulation of cellular process	3	73 out of 240 genes, 30.4%	1208 out of 13900, 8.7%	3.12E-19	0.00%
GO:0048519	negative regulation of biological process	2	79 out of 240 genes, 32.9%	1422 out of 13900, 10.2%	6.41E-19	0.00%
GO:0009790	embryo development	5	49 out of 240 genes, 20.4%	544 out of 13900, 3.9%	7.70E-19	0.00%
GO:0009987	cellular process	2	197 out of 240 genes, 82.1%	7327 out of 13900, 52.7%	1.14E-18	0.00%
GO:0009889	regulation of biosynthetic process	4	74 out of 240 genes, 30.8%	1307 out of 13900, 9.4%	7.12E-18	0.00%
GO:0007389	pattern specification process	5	46 out of 240 genes, 19.2%	501 out of 13900, 3.6%	7.83E-18	0.00%
GO:0051128	regulation of cellular component organization	4	56 out of 240 genes, 23.3%	777 out of 13900, 5.6%	2.56E-17	0.00%
GO:0050793	regulation of developmental process	3	53 out of 240 genes, 22.1%	701 out of 13900, 5.0%	3.89E-17	0.00%
GO:0003006	developmental process involved in reproduction	3	57 out of 240 genes, 23.8%	829 out of 13900, 5.9%	1.03E-16	0.00%
GO:0040011	locomotion	2	50 out of 240 genes, 20.8%	638 out of 13900, 4.6%	1.21E-16	0.00%
GO:0051252	regulation of RNA metabolic process	5	68 out of 240 genes, 28.3%	1225 out of 13900, 8.8%	1.39E-15	0.00%
GO:0050896	response to stimulus	2	112 out of 240 genes, 46.7%	2984 out of 13900, 21.5%	2.91E-15	0.00%
GO:0016070	RNA metabolic process	6	84 out of 240 genes, 35.0%	1825 out of 13900, 13.1%	3.67E-15	0.00%
GO:2000026	regulation of multicellular organismal development	5	44 out of 240 genes, 18.3%	534 out of 13900, 3.8%	4.18E-15	0.00%
GO:0051239	regulation of multicellular organismal process	3	51 out of 240 genes, 21.3%	723 out of 13900, 5.2%	4.61E-15	0.00%
GO:0000904	cell morphogenesis involved in differentiation	6	41 out of 240 genes, 17.1%	464 out of 13900, 3.3%	5.69E-15	0.00%
GO:0003002	regionalization	6	41 out of 240 genes, 17.1%	464 out of 13900, 3.3%	5.69E-15	0.00%
GO:0090304	nucleic acid metabolic process	5	89 out of 240 genes, 37.1%	2039 out of 13900, 14.7%	6.88E-15	0.00%
GO:0023052	signaling	2	81 out of 240 genes, 33.8%	1768 out of 13900, 12.7%	2.62E-14	0.00%
GO:0009887	animal organ morphogenesis	7	50 out of 240 genes, 20.8%	727 out of 13900, 5.2%	3.02E-14	0.00%
GO:0010629	negative regulation of gene expression	6	45 out of 240 genes, 18.8%	599 out of 13900, 4.3%	5.77E-14	0.00%
GO:0007154	cell communication	3	81 out of 240 genes, 33.8%	1804 out of 13900, 12.9%	8.75E-14	0.00%
GO:0010467	gene expression	5	91 out of 240 genes, 37.9%	2250 out of 13900, 16.2%	3.50E-13	0.00%
GO:0010608	posttranscriptional regulation of gene expression	7	26 out of 240 genes, 10.8%	200 out of 13900, 1.4%	1.29E-12	0.00%
GO:0007165	signal transduction	4	71 out of 240 genes, 29.6%	1525 out of 13900, 10.9%	2.49E-12	0.00%
GO:0051704	multi-organism process	2	69 out of 240 genes, 28.8%	1453 out of 13900, 10.5%	2.66E-12	0.00%
GO:0051716	cellular response to stimulus	3	82 out of 240 genes, 34.2%	1964 out of 13900, 14.1%	3.78E-12	0.00%
GO:0042330	taxis	4	32 out of 240 genes, 13.3%	339 out of 13900, 2.4%	6.05E-12	0.00%
GO:0030030	cell projection organization	4	44 out of 240 genes, 18.3%	650 out of 13900, 4.7%	6.25E-12	0.00%
GO:0006417	regulation of translation	8	21 out of 240 genes, 8.8%	132 out of 13900, 0.9%	1.42E-11	0.00%
GO:0010646	regulation of cell communication	4	49 out of 240 genes, 20.4%	819 out of 13900, 5.9%	1.70E-11	0.00%
GO:0065008	regulation of biological quality	3	62 out of 240 genes, 25.8%	1256 out of 13900, 9.0%	1.95E-11	0.00%
GO:0023051	regulation of signaling	4	49 out of 240 genes, 20.4%	825 out of 13900, 5.9%	2.26E-11	0.00%
GO:0048749	compound eye development	9	32 out of 240 genes, 13.3%	357 out of 13900, 2.6%	2.62E-11	0.00%
GO:0046483	heterocycle metabolic process	4	92 out of 240 genes, 38.3%	2458 out of 13900, 17.7%	3.04E-11	0.00%

GO:0007163	establishment or maintenance of cell polarity	3	25 out of 240 genes, 10.4%	208 out of 13900, 1.5%	3.04E-11	0.00%
GO:0035220	wing disc development	8	32 out of 240 genes, 13.3%	362 out of 13900, 2.6%	3.87E-11	0.00%
GO:0044271	cellular nitrogen compound biosynthetic process	5	78 out of 240 genes, 32.5%	1883 out of 13900, 13.5%	3.88E-11	0.00%
GO:0035295	tube development	5	44 out of 240 genes, 18.3%	686 out of 13900, 4.9%	4.31E-11	0.00%
GO:0006725	cellular aromatic compound metabolic process	4	93 out of 240 genes, 38.8%	2520 out of 13900, 18.1%	4.84E-11	0.00%
GO:0034248	regulation of cellular amide metabolic process	6	23 out of 240 genes, 9.6%	175 out of 13900, 1.3%	5.09E-11	0.00%
GO:0048583	regulation of response to stimulus	4	52 out of 240 genes, 21.7%	940 out of 13900, 6.8%	5.29E-11	0.00%
GO:1901360	organic cyclic compound metabolic process	4	94 out of 240 genes, 39.2%	2577 out of 13900, 18.6%	6.69E-11	0.00%
GO:0007423	sensory organ development	7	36 out of 240 genes, 15.0%	472 out of 13900, 3.4%	7.66E-11	0.00%
GO:0061564	axon development	12	30 out of 240 genes, 12.5%	326 out of 13900, 2.3%	9.25E-11	0.00%
GO:0000003	reproduction	2	66 out of 240 genes, 27.5%	1448 out of 13900, 10.4%	9.76E-11	0.00%
GO:0044260	cellular macromolecule metabolic process	4	108 out of 240 genes, 45.0%	3249 out of 13900, 23.4%	1.43E-10	0.00%
GO:0009791	post-embryonic development	5	40 out of 240 genes, 16.7%	600 out of 13900, 4.3%	2.19E-10	0.00%
GO:0021700	developmental maturation	3	25 out of 240 genes, 10.4%	227 out of 13900, 1.6%	2.31E-10	0.00%
GO:0031328	positive regulation of cellular biosynthetic process	5	36 out of 240 genes, 15.0%	496 out of 13900, 3.6%	3.43E-10	0.00%
GO:0007166	cell surface receptor signaling pathway	6	43 out of 240 genes, 17.9%	700 out of 13900, 5.0%	3.92E-10	0.00%
GO:0009059	macromolecule biosynthetic process	5	76 out of 240 genes, 31.7%	1890 out of 13900, 13.6%	4.64E-10	0.00%
GO:0040007	growth	2	33 out of 240 genes, 13.8%	423 out of 13900, 3.0%	5.16E-10	0.00%
GO:0048469	cell maturation	6	22 out of 240 genes, 9.2%	179 out of 13900, 1.3%	7.63E-10	0.00%
GO:0009994	oocyte differentiation	8	21 out of 240 genes, 8.8%	163 out of 13900, 1.2%	1.04E-09	0.00%
GO:0034641	cellular nitrogen compound metabolic process	4	97 out of 240 genes, 40.4%	2864 out of 13900, 20.6%	2.21E-09	0.00%
GO:0006928	movement of cell or subcellular component	3	42 out of 240 genes, 17.5%	714 out of 13900, 5.1%	3.26E-09	0.00%
GO:0002064	epithelial cell development	7	29 out of 240 genes, 12.1%	350 out of 13900, 2.5%	3.57E-09	0.00%
GO:0048569	post-embryonic animal organ development	7	32 out of 240 genes, 13.3%	442 out of 13900, 3.2%	9.07E-09	0.00%
GO:0048737	imaginal disc-derived appendage development	6	27 out of 240 genes, 11.3%	320 out of 13900, 2.3%	1.42E-08	0.00%
GO:0022607	cellular component assembly	4	56 out of 240 genes, 23.3%	1234 out of 13900, 8.9%	1.61E-08	0.00%
GO:0048589	developmental growth	3	28 out of 240 genes, 11.7%	348 out of 13900, 2.5%	1.78E-08	0.00%
GO:0048736	appendage development	5	27 out of 240 genes, 11.3%	325 out of 13900, 2.3%	2.03E-08	0.00%
GO:0032774	RNA biosynthetic process	6	55 out of 240 genes, 22.9%	1210 out of 13900, 8.7%	2.44E-08	0.00%
GO:0006996	organelle organization	4	73 out of 240 genes, 30.4%	1940 out of 13900, 13.9%	4.30E-08	0.00%
GO:0044085	cellular component biogenesis	3	59 out of 240 genes, 24.6%	1382 out of 13900, 9.9%	4.77E-08	0.00%
GO:0043170	macromolecule metabolic process	4	122 out of 240 genes, 50.8%	4256 out of 13900, 30.6%	5.70E-08	0.00%
GO:0042221	response to chemical	3	53 out of 240 genes, 22.1%	1163 out of 13900, 8.4%	5.71E-08	0.00%
GO:0007010	cytoskeleton organization	5	37 out of 240 genes, 15.4%	622 out of 13900, 4.5%	5.84E-08	0.00%
GO:0009605	response to external stimulus	3	47 out of 240 genes, 19.6%	950 out of 13900, 6.8%	5.92E-08	0.00%
GO:0007552	metamorphosis	5	31 out of 240 genes, 12.9%	449 out of 13900, 3.2%	6.73E-08	0.00%
GO:0030855	epithelial cell differentiation	6	29 out of 240 genes, 12.1%	399 out of 13900, 2.9%	8.85E-08	0.00%
GO:0048646	anatomical structure formation involved in morphogenesis	5	34 out of 240 genes, 14.2%	542 out of 13900, 3.9%	9.65E-08	0.00%
GO:0009058	biosynthetic process	3	83 out of 240 genes, 34.6%	2446 out of 13900, 17.6%	2.24E-07	0.00%
GO:0048707	instar larval or pupal morphogenesis	6	29 out of 240 genes, 12.1%	422 out of 13900, 3.0%	3.37E-07	0.00%
GO:0051246	regulation of protein metabolic process	6	35 out of 240 genes, 14.6%	602 out of 13900, 4.3%	3.92E-07	0.00%
GO:0048599	oocyte development	9	17 out of 240 genes, 7.1%	141 out of 13900, 1.0%	5.08E-07	0.00%
GO:0016071	mRNA metabolic process	7	27 out of 240 genes, 11.3%	375 out of 13900, 2.7%	5.29E-07	0.00%
GO:0018130	heterocycle biosynthetic process	5	59 out of 240 genes, 24.6%	1471 out of 13900, 10.6%	5.77E-07	0.00%
GO:1901362	organic cyclic compound biosynthetic process	5	60 out of 240 genes, 25.0%	1521 out of 13900, 10.9%	7.54E-07	0.00%
GO:0050808	synapse organization	4	23 out of 240 genes, 9.6%	283 out of 13900, 2.0%	1.13E-06	0.00%
GO:0044237	cellular metabolic process	3	130 out of 240 genes, 54.2%	4871 out of 13900, 35.0%	1.23E-06	0.00%
GO:0009968	negative regulation of signal transduction	5	24 out of 240 genes, 10.0%	313 out of 13900, 2.3%	1.54E-06	0.00%
GO:0001700	embryonic development via the syncytial blastoderm	5	18 out of 240 genes, 7.5%	172 out of 13900, 1.2%	1.57E-06	0.00%
GO:0030029	actin filament-based process	3	23 out of 240 genes, 9.6%	288 out of 13900, 2.1%	1.59E-06	0.00%
GO:0009792	embryo development ending in birth or egg hatching	6	19 out of 240 genes, 7.9%	194 out of 13900, 1.4%	1.65E-06	0.00%
GO:0019438	aromatic compound biosynthetic process	5	58 out of 240 genes, 24.2%	1473 out of 13900, 10.6%	1.73E-06	0.00%
GO:0044238	primary metabolic process	3	131 out of 240 genes, 54.6%	4956 out of 13900, 35.7%	1.95E-06	0.00%
GO:0030036	actin cytoskeleton organization	4	22 out of 240 genes, 9.2%	272 out of 13900, 1.9%	2.95E-06	0.00%
GO:0050890	cognition	5	17 out of 240 genes, 7.1%	159 out of 13900, 1.1%	3.34E-06	0.00%
GO:0007611	learning or memory	3	17 out of 240 genes, 7.1%	159 out of 13900, 1.1%	3.34E-06	0.00%
GO:0007267	cell-cell signaling	4	30 out of 240 genes, 12.5%	496 out of 13900, 3.6%	3.40E-06	0.00%
GO:1903311	regulation of mRNA metabolic process	8	15 out of 240 genes, 6.3%	120 out of 13900, 0.9%	3.46E-06	0.00%
GO:0051674	localization of cell	3	25 out of 240 genes, 10.4%	359 out of 13900, 2.6%	4.95E-06	0.00%
GO:0051179	localization	2	75 out of 240 genes, 31.3%	2241 out of 13900, 16.1%	5.13E-06	0.00%
GO:0010648	negative regulation of cell communication	4	24 out of 240 genes, 10.0%	334 out of 13900, 2.4%	5.62E-06	0.00%
GO:0023057	negative regulation of signaling	3	24 out of 240 genes, 10.0%	334 out of 13900, 2.4%	5.62E-06	0.00%
GO:0006807	nitrogen compound metabolic process	3	125 out of 240 genes, 52.1%	4755 out of 13900, 34.2%	1.16E-05	0.00%

GO:0001738	morphogenesis of a polarized epithelium	7	14 out of 240 genes, 5.8%	115 out of 13900, 0.8%	1.70E-05	0.00%
GO:0007610	behavior	2	32 out of 240 genes, 13.3%	596 out of 13900, 4.3%	1.73E-05	0.00%
GO:0051130	positive regulation of cellular component organization	4	21 out of 240 genes, 8.8%	274 out of 13900, 1.9%	1.82E-05	0.00%
GO:0006402	mRNA catabolic process	8	12 out of 240 genes, 5.0%	81 out of 13900, 0.6%	2.07E-05	0.00%
GO:0097305	response to alcohol	5	14 out of 240 genes, 5.8%	123 out of 13900, 0.9%	4.09E-05	0.00%
GO:0007164	establishment of tissue polarity	5	13 out of 240 genes, 5.4%	104 out of 13900, 0.7%	4.18E-05	0.00%
GO:0071704	organic substance metabolic process	3	134 out of 240 genes, 55.8%	5344 out of 13900, 38.4%	4.43E-05	0.00%
GO:0060828	regulation of canonical Wnt signaling pathway	8	11 out of 240 genes, 4.6%	70 out of 13900, 0.5%	4.48E-05	0.00%
GO:0035556	intracellular signal transduction	5	33 out of 240 genes, 13.8%	658 out of 13900, 4.7%	5.20E-05	0.00%
GO:0048585	negative regulation of response to stimulus	3	24 out of 240 genes, 10.0%	379 out of 13900, 2.7%	6.51E-05	0.00%
GO:0008283	cell proliferation	2	23 out of 240 genes, 9.6%	356 out of 13900, 2.6%	8.79E-05	0.00%
GO:0061061	muscle structure development	4	18 out of 240 genes, 7.5%	222 out of 13900, 1.6%	8.94E-05	0.00%
GO:0019827	stem cell population maintenance	4	12 out of 240 genes, 5.0%	92 out of 13900, 0.7%	8.96E-05	0.00%
GO:0098727	maintenance of cell number	3	12 out of 240 genes, 5.0%	92 out of 13900, 0.7%	8.96E-05	0.00%
GO:0007420	brain development	8	13 out of 240 genes, 5.4%	114 out of 13900, 0.8%	0.0001272	0.00%
GO:0044087	regulation of cellular component biogenesis	4	21 out of 240 genes, 8.8%	309 out of 13900, 2.2%	0.000145787	0.00%
GO:0032879	regulation of localization	3	24 out of 240 genes, 10.0%	399 out of 13900, 2.9%	0.000171217	0.00%
GO:0040029	regulation of gene expression, epigenetic	7	16 out of 240 genes, 6.7%	185 out of 13900, 1.3%	0.000205535	0.00%
GO:0033043	regulation of organelle organization	5	24 out of 240 genes, 10.0%	408 out of 13900, 2.9%	0.000258958	0.00%
GO:0016334	establishment or maintenance of polarity of follicular epithelium	8	7 out of 240 genes, 2.9%	27 out of 13900, 0.2%	0.000435054	0.00%
GO:0007028	cytoplasm organization	4	10 out of 240 genes, 4.2%	70 out of 13900, 0.5%	0.000490334	0.00%
GO:0040040	thermosensory behavior	3	6 out of 240 genes, 2.5%	18 out of 13900, 0.13%	0.000609411	0.00%
GO:0045165	cell fate commitment	5	21 out of 240 genes, 8.8%	341 out of 13900, 2.5%	0.000756067	0.00%
GO:0007049	cell cycle	3	34 out of 240 genes, 14.2%	782 out of 13900, 5.6%	0.000939121	0.00%
GO:0060322	head development	4	13 out of 240 genes, 5.4%	136 out of 13900, 0.9%	0.001015117	0.00%
GO:0009628	response to abiotic stimulus	3	24 out of 240 genes, 10.0%	440 out of 13900, 3.2%	0.001023349	0.00%
GO:0048584	positive regulation of response to stimulus	3	24 out of 240 genes, 10.0%	440 out of 13900, 3.2%	0.001023349	0.00%
GO:1905114	cell surface receptor signaling pathway involved in cell-cell signaling	5	12 out of 240 genes, 5.0%	119 out of 13900, 0.9%	0.00155411	0.00%
GO:0008298	intracellular mRNA localization	5	10 out of 240 genes, 4.2%	79 out of 13900, 0.6%	0.001554466	0.00%
GO:0007416	synapse assembly	7	14 out of 240 genes, 5.8%	165 out of 13900, 1.2%	0.001607909	0.00%
GO:0050684	regulation of mRNA processing	9	11 out of 240 genes, 4.6%	101 out of 13900, 0.7%	0.002054777	0.00%
GO:0007155	cell adhesion	3	15 out of 240 genes, 6.3%	194 out of 13900, 1.4%	0.002149062	0.00%
GO:0016333	morphogenesis of follicular epithelium	7	8 out of 240 genes, 3.3%	48 out of 13900, 0.3%	0.002293308	0.00%
GO:0048190	wing disc dorsal/ventral pattern formation	8	8 out of 240 genes, 3.3%	48 out of 13900, 0.3%	0.002293308	0.00%
GO:0022610	biological adhesion	2	15 out of 240 genes, 6.3%	196 out of 13900, 1.4%	0.002445133	0.00%
GO:0198738	cell-cell signaling by wnt	5	12 out of 240 genes, 5.0%	125 out of 13900, 0.9%	0.00263362	0.00%
GO:0010647	positive regulation of cell communication	4	21 out of 240 genes, 8.8%	369 out of 13900, 2.7%	0.002710748	0.00%
GO:0023056	positive regulation of signaling	3	21 out of 240 genes, 8.8%	369 out of 13900, 2.7%	0.002710748	0.00%
GO:0046677	response to antibiotic	4	10 out of 240 genes, 4.2%	85 out of 13900, 0.6%	0.003084082	0.00%
GO:0045995	regulation of embryonic development	6	10 out of 240 genes, 4.2%	85 out of 13900, 0.6%	0.003084082	0.00%
GO:0003008	system process	3	32 out of 240 genes, 13.3%	753 out of 13900, 5.4%	0.00340709	0.00%
GO:0008152	metabolic process	2	135 out of 240 genes, 56.3%	5763 out of 13900, 41.5%	0.003584107	0.00%
GO:0045475	locomotor rhythm	5	9 out of 240 genes, 3.8%	69 out of 13900, 0.5%	0.004237786	0.01%
GO:0097435	supramolecular fiber organization	4	16 out of 240 genes, 6.7%	233 out of 13900, 1.7%	0.004547182	0.01%
GO:0051641	cellular localization	3	38 out of 240 genes, 15.8%	995 out of 13900, 7.2%	0.004585394	0.01%
GO:0007417	central nervous system development	7	17 out of 240 genes, 7.1%	263 out of 13900, 1.9%	0.005049161	0.01%
GO:0051301	cell division	3	17 out of 240 genes, 7.1%	269 out of 13900, 7.2%	0.006840194	0.01%
GO:0044267	cellular protein metabolic process	5	60 out of 240 genes, 25.0%	1966 out of 13900, 14.1%	0.007712954	0.01%
GO:0007224	smoothened signaling pathway	6	8 out of 240 genes, 3.3%	58 out of 13900, 0.4%	0.010040063	0.01%
GO:0031047	gene silencing by RNA	4	9 out of 240 genes, 3.8%	77 out of 13900, 0.6%	0.010700696	0.01%
GO:1901700	response to oxygen-containing compound	4	19 out of 240 genes, 7.9%	341 out of 13900, 2.5%	0.01164929	0.01%
GO:0017145	stem cell division	4	10 out of 240 genes, 4.2%	99 out of 13900, 0.7%	0.012433405	0.01%
GO:0090130	tissue migration	3	13 out of 240 genes, 5.4%	171 out of 13900, 1.2%	0.013235904	0.01%
GO:0007268	chemical synaptic transmission	8	17 out of 240 genes, 7.1%	283 out of 13900, 2.0%	0.013422348	0.01%
GO:0043484	regulation of RNA splicing	7	10 out of 240 genes, 4.2%	101 out of 13900, 0.7%	0.014877967	0.01%
GO:0010033	response to organic substance	4	25 out of 240 genes, 10.4%	557 out of 13900, 4.0%	0.019237455	0.01%
GO:0007167	enzyme linked receptor protein signaling pathway	6	16 out of 240 genes, 6.7%	263 out of 13900, 1.9%	0.021210771	0.01%

Upregulated Go Terms						
GO_ID	Term	GO Hierarchy Level	CLUSTER FREQUENCY	GENOME FREQUENCY	CORRECTED_PVALUE	FDR_RATE
GO:0009636	response to toxic substance	4	10 out of 104 genes, 9.6%	148 out of 13900, 1.1%	5.59E-05	0.00%
GO:0051186	cofactor metabolic process	4	12 out of 104 genes, 11.5%	247 out of 13900, 1.8%	0.000112806	0.00%
GO:0051085	chaperone mediated protein folding requiring cofactor	5	5 out of 104 genes, 4.8%	24 out of 13900, 0.2%	0.000294892	0.00%
GO:0061077	chaperone-mediated protein folding	4	6 out of 104 genes, 5.8%	44 out of 13900, 0.3%	0.000310378	0.00%
GO:0006790	sulfur compound metabolic process	4	9 out of 104 genes, 8.7%	159 out of 13900, 1.1%	0.001060429	0.00%
GO:0009408	response to heat	4	7 out of 104 genes, 6.7%	88 out of 13900, 0.6%	0.001509776	0.00%
GO:0006986	response to unfolded protein	6	5 out of 104 genes, 4.8%	36 out of 13900, 0.3%	0.002435656	0.00%
GO:0009266	response to temperature stimulus	4	8 out of 104 genes, 7.7%	144 out of 13900, 1.0%	0.004440108	0.17%
GO:0035966	response to topologically incorrect protein	5	5 out of 104 genes, 4.8%	46 out of 13900, 0.3%	0.008346132	0.27%
GO:0008340	determination of adult lifespan	5	8 out of 104 genes, 7.7%	161 out of 13900. 1.2%	0.009976106	0.25%
GO:0035080	heat shock-mediated polytene chromosome puffing	5	3 out of 104 genes, 2.9%	9 out of 13900, 0.1%	0.012074269	0.22%
GO:0046680	response to DDT	5	3 out of 104 genes, 2.9%	9 out of 13900, 0.1%	0.012074269	0.21%
GO:0007568	aging	3	8 out of 104 genes, 7.7%	169 out of 13900, 1.2%	0.014118762	0.30%
GO:0035079	polytene chromosome puffing	6	3 out of 104 genes, 2.9%	10 out of 13900, 0.1%	0.017155109	0.38%
GO:0006979	response to oxidative stress	4	7 out of 104 genes, 6.7%	132 out of 13900, 0.9%	0.021427666	0.64%
GO:0055114	oxidation-reduction process	3	14 out of 104 genes, 13.5%	571 out of 13900, 4.1%	0.031921597	0.61%
GO:0001666	response to hypoxia	6	5 out of 104 genes, 4.8%	62 out of 13900, 0.4%	0.035838429	0.67%
GO:0042221	response to chemical	3	21 out of 104 genes, 20.2%	1163 out of 13900, 8.4%	0.043897762	0.80%

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VITA

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At Loyola, Alex was elected secretary of the biology graduate school association and graduated with Magna Cum Laude honors from 2016 to 2018. He also presented research at two symposiums and spoke at the Midwest Drosophila Conference.

Going forward, Alex will be attending the University of Notre Dame in pursuit of a Ph.D. in biological sciences. Upon arrival, he plans to conduct research focused primarily on cancer biology.